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A comparative study of some wilt-producing phytopathogenic bacteria

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A COMPARATIVE STUDY OF SOME WILT-PRODUCING PHYTOPATHOGENIC BACTERIA

by

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INTRODUCTION

Among the phytopathogenic bacteria, nine species are known to cause vascular necrosis. These vascular parasites do not constitute a taxonomic group like the soft-rot, green-fluorescent, and gall producing organisms but are actually a very heterogeneous group taxonomically. In their cultural reactions they can be separated into two groups that have several reactions in common. These organisms are as follows: Xanthomonas campestris in cabbage, X. lespedezae in species of Lespedeza, Corynebacterium flaccumfaciens in common beans, C. michiganensis in tomatoes, C. sepedonicum in Irish potatoes, C. insidiosum in alfalfa, Erwinia tracheiphila in cucumbers, Pseudomonas solanacearum in members of the Solanaceae, and Bacterium stewartii in maize.

No attempt has been made yet to study these organisms comparatively as two groups of vascular parasites although some of them have been studied culturally in detail. Therefore it was hoped that a comparative study of their growth response might shed new light on their one common characteristic, their host-parasite relationship. The present study comprises two phases of the activity of these bacteria, namely their growth response and parasitism.

SOURCE OF CULTURES

The source of the isolates used in the present work is given in table 1. Three or more isolates of each organism were obtained except Corynebacterium sepedonicum and Erwinia tracheiphila where one isolate of each species was used.

Table 1. Source of cultures.

Culture No.	Species	From whom received	Host and date of isolation
H-cl	Xanthomonas campestris	W. J. Hooker	cabbage, --
H-gar	"	"	" , --
XC-2	"	W. H. Burkholder	cauliflower, 1936
XC-13	"	"	" , "
XC-15	"	"	cabbage, 1944
19604	Xanthomonas lespedezae	C. L. Lefebvre	lespedeza, 1946
XL-1	"	W. H. Burkholder	" , 1945
XL-2	"	"	" , "
7392	Corynebacterium flaccumfaciens	Amer. Type Cult. Collect.	bean, --
2A	"	S. P. Doolittle	" , --
2B	"	"	" , --
CF-4	"	W. H. Burkholder	" , 1923
CF-14	"	"	23-year-old bean seed, 1945
7433	Corynebacterium michiganensis	Amer. Type Cult. Collect.	tomato, --
CM-1	"	W. H. Burkholder	" , 1936
CM-6	"	"	" , 1940
3-D	"	S. P. Doolittle	" , --
9850	Corynebacterium sepedonicum	Amer. Type Cult. Collect.	potato, --

Table 1. (continued)

Culture No.	Species	From whom received	Host and date of isolation
CI-13	Corynebacterium insidiosum	W. H. Burkholder	alfalfa, 1943
CI-15	"	"	" , "
2246	"	F. R. Jones	" , 1946
2247	"	"	" , "
2248	"	"	" , "
8199	Bacterium stewartii	Amer. Type Cult. Collect.	sweet corn, --
Linc.500	"	A. J. Riker	" , --
46-D-35	"	S. P. Doolittle	" , --
SS-1	"	W. H. Burkholder	" , 1932
SS-12	"	"	" , 1941
9910	Pseudomonas solanacearum	Amer. Type	-- , --
16-a	"	J. H. Jensen	potato, 1946
16-a-I_2	"	"	" , "
9911	Erwinia tracheiphila	Amer. Type Cult. Collect.	cucumber, --

Before the study was begun, the cultures were tested for their purity and pathogenicity, and maintained on nutrient dextrose agar; exceptions to this were Corynebacterium sepedonicum and Erwinia tracheiphila, which were maintained on potato dextrose agar.

Note. The writer is deeply indebted to the various persons who supplied cultures to him. Their addresses are: Dr. W. J. Hooker, Botany Department, Iowa State College, Ames, Iowa; Dr. W. H. Burkholder, Department of Plant Pathology, Cornell University, Ithaca, N. Y.; Dr. C. L. Lefebvre and Dr. S. P. Doolittle, U. S. Department of Agriculture, Bureau of Plant Industry, Beltsville, Md.; Dr. F. R. Jones and Dr. A. J. Riker, Department of Plant Pathology, University of Wisconsin, Madison, Wis.; and Dr. J. H. Jensen, North Carolina State College, Raleigh, N. C.

GROWTH REACTION AND CULTURAL RESPONSE OF THE WILT BACTERIA

Morphology and Staining Reactions

The morphological characters of the bacterial species studied are well established and therefore no attempt was made to make a detailed study of them. All the organisms were rod shaped, did not form any spores, and were not acid fast.

Motility.

Motility was determined by growing the isolates on semi-solid agar as recommended in the Manual of Methods (1936). All the isolates of Pseudomonas solanacearum, Xanthomonas campestris, X. lespedezae, Corynebacterium flaccumfaciens, and Erwinia tracheiphila were motile, whereas the remaining species were non-motile. As a check, a motile strain of Escherichia coli was included in the study.

Gram's stain.

Both young and old cultures of all the isolates were stained by the Hucker modification of Gram's stain (Manual of Methods, 1936). All the Corynebacteria were Gram positive, while the rest of the species were Gram negative. Smears of Bacillus subtilis (a Gram positive organism) stained at the same time were Gram positive.

Cultural Characters

The cultural characters of the organisms were studied on various media which were prepared according to standard procedures using Difco ingredients. The cultures were incubated at 25° C. unless otherwise mentioned. The media employed were: nutrient agar, nutrient broth, nutrient dextrose agar, potato dextrose agar, and potato plugs. The colony characters were studied on nutrient dextrose agar plates.

Nutrient agar slants.

In general, growth was poor on this medium. The predominant color of the cultures was some shade of yellow except in the case of Pseudomonas solanacearum, which was dirty white to brown. Erwinia tracheiphila failed to grow and all cultures of Corynebacterium insidiosum grew scantily. All isolates of C. flaccumfaciens and C. michiganensis grew moderately well on this medium. Corynebacterium sepedonicum grew rather poorly. The isolates of Bacterium stewartii were differentiated into two groups on the basis of their growth on this medium; three of the five isolates grew rather well and produced good, slimy growth. The remaining two isolates grew poorly and showed dull, granular growth. Xanthomonas campestris and X. lespedezae grew moderately well and were indistinguishable.

Nutrient broth.

The results on this medium showed the same trends as on nutrient agar. Appreciable turbidity was produced by Pseudomonas solanacearum which also produced a distinct pellicle. No growth of Erwinia tracheiphila was apparent even after two weeks. All the rest of the isolates showed slight turbidity, slight ring, and a flocculent sediment after eight days. The color of the medium remained unchanged and odor was absent in all cases.

Nutrient dextrose agar slants.

This medium was prepared by adding one per cent dextrose to nutrient agar, and was found to be very suitable for growth of the organisms; Erwinia tracheiphila was the only exception and grew very poorly. Xanthomonas camnestris, X. lespedezae, and Bacterium stewartii were brilliant yellow; some isolates of Corynebacterium flaccumfaciens were yellow and some were pink, but this was not a stable character. All isolates of C. michiganensis were dull yellow. Corynebacterium sepedonicum was white on this medium and Pseudomonas solanacearum was dirty white turning brown. Corynebacterium insidiosum was yellow in young cultures but turned slightly blue on aging. All the isolates of this species did not, however, behave uniformly in this respect. The isolates of Bacterium stewartii varied as regards growth on this medium. Three isolates of

the five produced yellow, slimy growth while the other two showed a dark yellow, dull growth; apparently the first three isolates, Type, Linc·500, and 46-D-35 belong to Type B cultures of Bacterium stewartii described by Ivanoff, Riker and Dettwiler (1938) and the remaining two, SS-1 and SS-12, belong to Type A.

Potato dextrose agar slants.

All species grew copiously on this medium, even Erwinia tracheiphila. Growth and pigment production were the same as on nutrient dextrose agar, except that the pigment was more intense, and growth was more abundant.

Potato plugs.

All the isolates grew well on this medium and produced intense colors. Pseudomonas solanacearum was dark brown on this medium and most of the isolates of Corynebacterium insidiosum were blue-black. The potato plug was discolored in most cases.

Colonies on nutrient dextrose agar plates.

Colonies of almost all the isolates were round, with entire margins, and no particular markings. Seven day old colonies ranged in diameter size from 10 to 12 mm. in the case of Xanthomonas campestris and X. lespedezae to two to three mm. in Erwinia tracheiphila. The rest of the species were intermediate in size.

The Influence of Temperature on Growth of the Organisms

The effect of temperature on the growth of the organisms was determined by growing them at different temperatures. Single streak inoculations were made on slants of nutrient dextrose agar which were then incubated at 15°, 20°, 25°, 30°, 37°, and 40° C. respectively. Potato dextrose agar slants were used in the case of Erwinia tracheiphila and Corynebacterium sepedonicum since these species do not grow well on the other medium. Duplicate cultures were carried at each temperature and the amount of growth in each case was measured in an arbitrary manner at the end of one, three, and five days. Table 2 gives the results of this trial.

Table 2. Influence of temperature on growth.

Temperature	15°C.			20°C.			25°C.			30°C.			37°C.			40°C.		
Incubation days	1	3	5	1	3	5	1	3	5	1	3	5	1	3	5	1	3	5
Organism	Vigor of growth																	
Xanthomonas campestris, H-cl	0	3	4	1	3	4	2	4	4	3	4	4	3	4	4	0	1	1
" , H-gor	1	3	4	1	3	4	2	4	4	3	4	4	3	4	4	0	1	1
" , XC-2	1	3	4	1	3	4	2	4	4	3	4	4	2	4	4	0	1	1
" , XC-13	0	3	4	2	3	4	1	4	4	2	4	4	2	4	4	0	1	1
" , XC-15	0	3	4	2	4	4	2	4	4	1	4	4	3	4	4	0	1	1
Xanthomonas lespedezae, 19604	0	3	4	3	4	4	2	4	4	3	4	4	2	4	4	1	3	3
" , XL-1	0	3	4	3	4	4	2	4	4	3	4	4	2	4	4	1	3	3
" , XL-2	0	3	4	2	4	4	3	4	4	2	4	4	2	4	4	1	3	3

Table 2. (continued)

Temperature	15°C.			20°C.			25°C.			30°C.			37°C.			40°C.		
Incubation days	1	3	5	1	3	5	1	3	5	1	3	5	1	3	5	1	3	5
Organism	Vigor of growth																	
Corynebacterium flaccumfaciens, 7392	0	2	3	0	1	4	0	2	3	1	2	3	1	2	3	0	1	2
" , 2A	0	3	3	0	2	3	2	3	3	1	2	3	1	3	3	0	2	3
" , 2B	0	2	3	0	3	4	0	2	3	1	3	3	1	2	3	0	2	3
" ,CF-4	0	1	2	0	2	3	0	2	3	0	1	2	1	2	3	0	2	3
" , CF-14	0	1	2	0	1	3	0	2	3	1	2	3	1	2	3	0	1	2
Corynebacterium michiganensis, 7433	1	2	3	0	1	3	2	3	3	1	2	3	2	2	2	0	0	0
" , CM-1	0	0	1	0	1	3	0	1	2	1	1	2	0	1	1	0	0	0
" , CM-6	0	1	1	0	1	2	1	1	3	1	2	2	0	1	1	0	0	0
" , 3-D	0	0	1	0	1	3	0	2	2	0	1	2	0	1	1	0	0	0
Corynebacterium sepedonicum, 9850	0	3	4	0	3	4	1	3	4	0	1	1	0	0	1	0	0	0
Corynebacterium insidiosum, CI-13	0	0	1	0	1	2	0	2	3	0	1	1	0	0	0	0	0	0
", CI-15	0	0	1	0	1	2	0	2	3	0	1	1	0	0	0	0	0	0
", 2246	0	0	2	0	1	2	0	2	3	0	1	1	0	0	0	0	0	0
", 2247	0	1	2	0	1	3	0	1	3	0	1	1	0	0	0	0	0	0
", 2248	0	1	2	0	0	1	0	1	2	0	1	1	0	0	0	0	0	0
Bacterium stewartii, 8199	0	2	4	2	4	4	3	4	4	4	4	4	3	3	4	2	2	3
" ", Linc.500	0	1	3	2	4	4	2	4	4	3	4	4	2	3	3	2	2	3
" ", 46-D-35	2	3	3	2	3	3	2	3	3	2	2	3	2	3	3	2	2	3
" ", SS-1	0	0	2	0	1	2	1	2	2	1	1	2	1	1	2	2	2	2
" ", SS-12	0	1	2	1	1	2	2	2	3	2	2	3	1	2	3	1	2	2
Pseudomonas solanacearum, 9910	0	0	1	0	1	3	1	3	4	2	4	4	2	4	4	1	2	3
" , 16-a	0	0	1	0	1	3	1	3	4	2	4	4	2	4	4	1	2	3
" , 16a-I_2	0	0	1	0	1	3	1	3	4	2	4	4	2	4	4	1	2	2
Erwinia tracheiphila, 9911	0	0	0	0	1	3	0	0	2	0	1	2	0	0	0	0	0	0

The results show that of the species of wilt-bacteria under study, Xanthomonas campestris, X. lespedezae, Pseudomonas solanacearum, and Bacterium stewartii have a wide growth-temperature range. Of the species of Corynebacterium, C. flaccumfaciens seems to be the only species having a wide range of temperature for growth, while the remaining three species, viz., C. michiganensis, C. sepedonicum, and C. insidiosum have very narrow limits of temperature for growth. The optimum for all the species of Corynebacterium seems to be between 20° and 25° C., and the same is true for Erwinia tracheiphila.

Liquefaction of Gelatin

The ability of the bacteria to liquefy gelatin was determined by growing them on three gelatin media: plain gelatin, nutrient gelatin, and Frazier's gelatin. Stab cultures were made on the first two media and plates of Frazier's medium were inoculated. Both plain and nutrient gelatin were neutralized to brom thymol blue, as gelatin is generally acidic. The plates and tubes were inoculated at 25° C. and observations were made after eight days in the case of the stab cultures and after forty-eight hours in the case of Frazier's gelatin medium. Liquefaction of gelatin on this last medium was determined by flooding the plates with an acidified solution of mercuric chloride and the zone of hydrolysis was recorded. The results are shown in table 3.

Table 3. Liquefaction of gelatin by the wilt-bacteria.

Organism	Plain Gelatin: Liquefaction	Nutrient Gelatin: Liquefaction	Nutrient Gelatin: Reaction	Frazier's Gelatin: Hydrolysis	Frazier's Gelatin: Zone mm.
Xanthomonas campestris, H-cl	+ + +	+ + +	alkaline	+ + +	26
" , H-gor	+ + +	+ + +	"	+ + +	30
" , XC-2	+ + +	+ + +	"	+ + +	23
" , XC-13	+ + +	+ + +	"	+ + +	20
" , XC-15	+ + +	+ + +	"	+ + +	21
Xanthomonas lespedezae, 19604	+ + + +	+ + + +	"	+ + + +	22
" , XL-1	+ + + +	+ + + +	"	+ + + +	28
" , XL-2	+ + + +	+ + + +	"	+ + + +	30
Corynebacterium flaccumfaciens, 7392	* --	+ +	"	+ +	10
" , 2A	* --	+ +	neutral	+ +	9
" , 2B	* --	-	"	-	-
" , CF-4	* --	-	"	-	-
" , CF-14	* --	+ +	alkaline	+ +	10
Corynebacterium michiganensis, 7433	* --	+ + +	neutral	+ +	11
" , CM-1	* --	+ +	"	+ +	10
" , CM-6	* --	+ +	alkaline	+	6
" , 3-D	* --	-	neutral	-	-
Corynebacterium sepedonicum, 9850	* --	-	"	-	-
Corynebacterium insidiosum, CI-13	* --	-	"	-	-
" , CI-15	* --	-	"	-	-
" , 2246	* --	-	"	-	-
" , 2247	* --	-	"	-	-
" , 2248	* --	-	"	-	-
Bacterium stewartii, 8199	* --	-	"	-	-
" " Linc.500	* --	-	"	-	-

Table 3. (continued)

Organism	Plain Gelatin: Lique-faction	Nutrient Gelatin: Lique-faction	Nutrient Gelatin: Reaction	Frazier's Gelatin: Hydrolysis	Frazier's Gelatin: Zone mm.
Bacterium stewartii, 46-D-35	* --	-	neutral	-	-
" , SS-1	* --	-	"	-	-
" , SS-12	* --	-	"	-	-
Pseudomonas solanacearum, 9910	* --	-	alkaline	-	-
" , 16-a	* --	-	"	-	-
" , 16-a-I_2	* --	-	"	-	-
Erwinia tracheiphila, 9910	* --	-	"	-	-

Note: * = no growth; - = no liquefaction;
+ = liquefaction

The results show that both Xanthomonas campestris and X. incanedezae are strong gelatin liquefiers and can even attack plain gelatin. Of the species of Corynebacterium, C. flaccumfaciens and C. michiganensis attack gelatin moderately. The different isolates of these two species varied in their ability to attack gelatin. All the remaining species seem to be unable to attack gelatin in eight days.

The results further indicate that more than one medium is necessary for the study of gelatin liquefaction. Some organisms cannot grow on plain gelatin and the use of this medium alone would give unreliable results. Frazier's gelatin medium seems to be the most satisfactory for study of gelatin liquefaction;

it gives quick results and the relative abilities of different organisms can also be measured in a quantitative manner by means of the zones of hydrolysis. This medium alone should be adequate enough to study gelatin liquefaction.

Hydrolysis of Starch

The production of amylase in culture was studied by growing the organisms in plates of nutrient agar containing 0.5 per cent potato starch. A thin paste of starch was made in a small quantity of nutrient broth and this was added to the bulk of the medium before sterilization. Plates were then poured and inoculated with the organisms. Four cultures were inoculated in each plate making stab inoculations with a straight needle. This method gave good, round colonies. After five days incubation at 25° C. the plates were flooded with a weak solution of iodine. A clear zone around the colony indicated starch digestion. The zones were measured wherever present. Table 4 shows the results.

The results show that only three species, viz., Xanthomonas campestris, X. lespedezae, and Corynebacterium sepedonicum are able to hydrolyze starch. The above results were confirmed by growing the organisms on nutrient broth containing 0.5 per cent potato starch and testing the medium for the presence or absence of it at the end of six days by addition of iodine solution. In the cultures of Xanthomonas campestris and X. lespedezae no

Table 4. Hydrolysis of starch by species of wilt bacteria.

Organism	Colony size, mm.	Zone of hydrolysis, mm.
Xanthomonas campestris, H-cl	15	30
" " , H-gor	16	35
" " , XC-2	9	25
" " , XC-13	12	16
" " , XC-15	4	11
Xanthomonas lespedezae, 19604	15	29
" , XL-1	15	27
" , XL-2	14	30
Corynebacterium flaccumfaciens, 7392	6	0
" , 2A	7	0
" , 2B	6	0
" , CF-4	6	0
" , CF-14	5	0
Corynebacterium michiganensis, 7433	4	0
" , CM-1	4	0
" , CM-6	6	0
" , 3-D	4	0
Corynebacterium sepedonicum, 9850	6	15
Corynebacterium insidiosum, CI-13	6	0
" , CI-15	6	0
" , 2246	5	0
" , 2247	4	0
" , 2248	4	0
Bacterium stewartii, 8199	22	0
" " , Linc. 500	16	0
" " , 46-D-35	18	0
" " , SS-1	5	0
" " , SS-12	4	0

Table 4. (continued)

Organism	Colony size, mm.	Zone of hydrolysis, mm.
Pseudomonas solanacearum, 9910	10	0
" , 16-a	11	0
" , 16-a-I_2	11	0
Erwinia tracheiphila, 9911	5	0

starch was found at the end of six days while in the culture of Corynebacterium sepedonicum partial hydrolysis of starch occurred.

Production of Hydrogen Sulphide and its Relation to Disease Symptoms

Two methods are generally employed for the detection of hydrogen sulphide in bacterial cultures. Some workers have used agar media containing salts of iron, lead or cobalt as indicators whilst others have employed liquid cultures over which lead acetate or lead carbonate impregnated strips are suspended. In the present work both methods were employed. The solid media used were Kligler's iron agar and the medium described by Vaughn and Levine (1936). This medium has the following composition:

Proteose peptone (Difco)	20.0 g.
Di-potassium hydrogen phosphate	1.0 "
Ferric citrate	0.5 "
Agar	15.0 "
Distilled water	1.0 litre

Slants of Kligler's iron agar were inoculated by streaking on the surface of the slant and stabbing the butt of the agar column as in the case of Russell's double sugar agar. The other medium was used in the form of stab cultures. The cultures on both the media were incubated at 25° C. and observations were made at the end of a week. None of the cultures showed any blackening of the medium, in either case.

As none of the cultures showed presence of hydrogen sulphide in the above test, cultures were made into nutrient broth containing 0.05 percent cystine hydrochloride. Strips of filter paper impregnated with a saturated solution of lead acetate, and dried in a steam oven after sterilization were suspended in the tubes over the medium and the cultures were then incubated at 25° C. Observations were madeevery day.

All the cultures of Xanthomonas campestris and X. lespedezae showed positive darkening of the filter paper after 24 hours incubation indicating production of hydrogen sulphide. After one week's incubation, all isolates except those of Pseudomonas solanacearum showed darkening of the filter paper; Erwinia tracheiphila showed feeble production of hydrogen sulphide.

As all the cultures except those of Pseudomonas solanacearum produced hydrogen sulphide in the above medium, another

set of cultures were made in nutrient broth without cystine and on nutrient broth and nutrient agar prepared with proteose peptone and one per cent dextrose. The filter paper method was used in every case for detection of hydrogen sulphide.

In one week, Xanthomonas campestris and X. lespedezae showed blackening of the filter paper strips on all media. None of the other species produced any hydrogen sulphide on both the liquid media; while on nutrient-dextrose-agar made up with proteose peptone, all the species except Pseudomonas solanacearum showed positive darkening of the filter paper. Isolates of Bacterium stewartii produced very feeble darkening on this medium but it was present, nevertheless. Uninoculated controls did not show any darkening. These results are summarized in table 5.

Of the species under study, positive hydrogen sulphide production is reported for Xanthomonas campestris and X. lespedezae, while Corynebacterium sepedonicum and Erwinia tracheiphila are reported as feeble producers of hydrogen sulphide (Bergey, et al, 1939). The rest of the species are reported as non-producers of hydrogen sulphide. In the present experiments, the only species that failed to produce hydrogen sulphide was Pseudomonas solanacearum. It seems that lead acetate strip is a better method of testing for hydrogen sulphide than use of lead or iron salts in the medium. It is interesting to note that all the wilt bacteria except Pseudomonas solanacearum produced hydrogen

Table 5. Production of hydrogen sulphide on various media.

Organism	No. of isolates	Production of H_2S on Nutr. broth (cystine)	Nutr. dext. broth (prot. peptone)	Nutr. dext. agar (prot. peptone)
Xanthomonas campestris	5	+	+	+
Xanthomonas lespedezae	3	+	+	+
Corynebacterium flaccumfaciens	5	+	-	+
Corynebacterium michiganensis	4	+	-	+
Corynebacterium sepedonicum	3	+	-	+
Corynebacterium insidiosum	5	+	-	+
Bacterium stewartii	5	+	-	+ (feeble)
Pseudomonas solanacearum	3	-	-	-
Erwinia tracheiphila	1	+	-	+

sulphide and this seems to be common character of these organisms.

It is also interesting to note that most of these pathogens produce blackening of the vascular system in the host plant. The exceptions are Bacterium stewartii and Erwinia tracheiphila, and these two species also produce very little hydrogen sulphide in culture. It was therefore thought worthwhile to find out whether hydrogen sulphide is responsible for production of blackening in the host tissue. Cabbage was selected for the experiments as cabbage affected with "black-rot" shows the most blackening amongst all the various hosts. The causal organism, Xanthomonas campestris, also produces large amounts of hydrogen sulphide.

The ability of Xanthomonas campestris to produce hydrogen sulphide from cabbage extract and various parts of the cabbage plant was first tested. Cabbage extract was prepared by boiling 200 gms. of macerated cabbage in a litre of water for 15 minutes. The extract was then used as a culture medium in test tubes. Small pieces of cabbage root, stem, and leaves were also used as a medium in test tubes. After sterilization, duplicate tubes of each medium were inoculated with a virulent culture of Xanthomonas campestris and strips of lead acetate impregnated filter paper were suspended over the inoculated tubes. Uninoculated tubes served as check. The cultures were incubated at 25° C. and observations were

made on alternate days.

The organism produced hydrogen sulphide from all the media in six days as evidenced by the blackening of the filter paper. These results show that the tissues of cabbage contain substances which give rise to hydrogen sulphide as a result of bacterial action.

The ability of pure hydrogen sulphide to produce blackening in the vascular system of cabbage was next investigated. A saturated water solution of hydrogen sulphide was prepared and dilutions were made from this stock solution; the dilutions used were full strength, one in 10, one in 100, and one in 1,000. These various solutions were placed in test tubes. Young cabbage seedlings, about a month old were then placed in these test tubes. In some cases, the roots were cut off whereas in other cases, entire seedlings were used. Leaves of older cabbage plants, with their petioles cut at the base were also used in this experiment. In another case, a strong, undiluted solution was injected in the petioles of leaves on an old cabbage plant hypodermically. The test tubes containing the seedlings and leaves were plugged with cotton wool and left on the laboratory bench near a window. Checks consisted of similar tubes where sterile water was used.

In 12 hours, the detached leaves kept in an undiluted solution of hydrogen sulphide had wilted. On examination of the base of the petiole, a blackening of the vascular system

was visible. Freehand sections were made and examined and these showed blackening of the xylem vessels. This blackening was not, however, present beyond a distance of about a centimeter from the base of the petiole. Similar results were obtained in the case of seedlings which had their roots cut off. Blackening was more restricted in this case and could not be traced beyond a distance of about two millimeters from the cut ends.

All the other dilutions failed to produce any blackening in the seedlings and leaves which remained turgid.

A small amount of discoloration was visible in the leaf petioles on an older plant where hydorgen sulphide was injected hypodermically. Freehand sections showed general blackening of the tissues along the path of the needle but the unwounded vessels were free from it. Inoculated leaves did not wilt.

These results, though not conclusive, indicate that hydrogen sulphide could be responsible for producing the characteristic blackening of the xylem vessels in cabbage plants invaded by Xanthomonas campestris. It is possible that the bacteria produce hydrogen sulphide as they progress through the xylem vessels, resulting in blackening. Further work in this connection would prove interesting.

Production of Indole

The ability of the organisms to produce indole was tested by growing them on nutrient broth containing 0.01 per cent tryptophane. Duplicate cultures were incubated at 25° C. in each case and the presence of indole was tested at the end of five and ten days with Kovac's reagent. An indole positive strain of Escherichia coli was used as control.

None of the cultures showed presence of indole at the end of five days whereas two cultures of X. campestris and all the cultures of X. lespedezae gave a positive test for indole at the end of 10 days. Bacterium stewartii, reported as a feeble producer of indole, did not give a positive test after 10 days growth.

Action on Litmus Milk

Duplicate cultures in litmus milk were incubated at 25° C. Observations were made at the end of 3,5, 10, 15, and 30 days.

A definite acid reaction was produced by all cultures of C. insidiosum but no coagulation was noticed; moderate reduction of litmus was evident after 14 days. Pseudomonas solanacearum produced a definite alkaline reaction. Erwinia tracheiphila produced no change in the milk. All cultures of Xanthomonas campestris and X. lespedezae showed complete proteolysis in 14 days. The former showed an alkaline reaction

while the latter showed an acid reaction in the beginning, which later disappeared. Corynebacterium flaccumfaciens produced complete proteolysis and moderate reduction of litmus in one month. Corynebacterium michiganensis curdled the milk without production of acid and reduced the litmus moderately. Corynebacterium sepedonicum showed slight reduction of the litmus. These results are summarized in table 6.

Reduction of Nitrates

The ability of the organisms to reduce nitrates to nitrites was determined in a preliminary way by growing them on nutrient broth containing 0.1 per cent potassium nitrate. The cultures were incubated at 25° C. for five days and the test for nitrite was made by the sulphanilic acid -α-napthylamine method (Manual of Methods, 1936). Of the species under study, only Pseudomonas solanacearum gave a positive test for nitrites in five days; all the cultures of this species also gave a positive test for nitrates when tested with zinc dust.

In another trial a synthetic semisolid nitrate medium containing 0.1 per cent agar was inoculated as recommended by Zobell (1932). On this medium only Xanthomonas campestris, X. lespedezae, Pseudomonas solanacearum and Bacterium stewartii grew well while Erwinia tracheiphila and the species of Corynebacterium failed to make any growth. At the end of five days,

Table 6. Action on litmus milk.

Organism	No. of isolates	Acid	Alkali	Curd	Proteolysis	Reduction
Xanthomonas campestris	5	-	+	-	+	-
Xanthomonas lespedezae	3	-	-	-	+	-
Corynebacterium flaccumfaciens	5	-	-	-	+	+
Corynebacterium michiganensis	4	-	-	+	-	+
Corynebacterium sepedonicum	1	-	-	-	-	+(slight)
Corynebacterium insidiosum	5	+ (slight)	-	-	-	+
Bacterium stewartii	5	+ (slight)	-	-	-	-
Pseudomonas solanacearum	3	-	+	-	-	-
Erwinia tracheiphila	1	-	-	-	-	-

only Pseudomonas solanacearum gave a positive test for nitrites whereas the two species of Xanthomonas and Bacterium stewartii showed absence of nitrites. To test whether nitrates were still present or otherwise, a pinch of zinc dust was added to each tube showing a negative nitrite reaction. All the tubes produced a bright red color on addition of zinc dust, showing thereby that the nitrates were not reduced beyond the nitrite stage.

Since Pseudomonas solanacearum was the only species that showed reduction of nitrates, it was thought advisable to find out whether this organism reduced nitrates beyond the nitrites stage. Tubes of the synthetic nitrate medium were inoculated and the cultures were tested for nitrites and nitrates at the end of 3, 8, 10, 15, 20 and 30 days incubation. No nitrites were produced till the cultures were 8 days old and nitrates were still present. Nitrites and nitrates were present after 10 and 15 days growth, but both disappeared after 20 days.

Of the species under study, Pseudomonas solanacearum is the only organism that reduces nitrates. It is also the only species which has a very wide host range distributed in a number of widely separated families. On the other hand, the remaining species of wilt bacteria have rather narrow limits of host range, the organisms in each case infecting hosts belonging to single families.

The Voges-Proskauer and Methyl-Red Tests

The ability of the organisms to produce acetyl-methyl-carbinol from dextrose (V-P test), and produce enough acidity to give a positive reaction with methyl red, was tested by growing the organisms in Difco-MRVP medium for five days at 25° C. At the end of this period, each culture was divided into two equal portions. One series of the cultures was tested for the V-P test by adding α-napthol and potassium hydroxide as recommended in the Manual of Methods (1936) and the other series was tested for acidity with methyl red. All cultures were negative for both these tests.

Citrate Utilization Test

The organisms were grown on Koser's citrate medium (Difco) for a period of five days at 25° C. to determine whether any of the species would utilize sodium citrate as the sole source of carbon. Of the species under study, Xanthomonas campestris, X. lespedezae and Pseudomonas solanacearum alone grew on this medium and produced a heavy turbidity in each case. The rest of the species, viz. Corynebacterium flaccumfaciens, C. michiganensis, C. sepedonicum, C. insidiosum, Erwinia tracheiphila and Bacterium stewartii failed to grow on this medium.

Growth on Synthetic Asparagin Medium

Starr and Weiss (1943), using a synthetic medium containing 0.5 per cent asparagin as the sole source of carbon and nitrogen found that only species of green fluorescent plant pathogens of the genus Pseudomonas could grow on this medium whereas species of Xanthomonas and of Corynebacterium failed to do so. In order to confirm these results with respect to the species of wilt bacteria under study, the organisms were grown on the medium using the serial transfer technique described by Starr and Weiss (ibid.). Only Pseudomonas solanacearum and Bacterium stewartii grew on this medium through three successive transfers while none of the other species showed any growth. These results confirm the findings of Starr and Weiss (ibid.) and this test appears to be quite satisfactory for generic differentiation between the plant pathogenic bacteria.

In another test, one per cent dextrose was added to the asparagin medium as an additional source of carbon and inoculations of all the isolates were made on plates of this medium. Xanthomonas campestris and X. lespedezae, Bacterium stewartii, and Pseudomonas solanacearum showed positive growth on this medium but none of the Corynebacterium species showed any growth.

It seems, therefore, that species of Xanthomonas can

utilize asparagin as a source of nitrogen but not of carbon. These results agree with those of Starr (1946).

Utilization of Carbon Compounds

The literature pertaining to the utilization of carbohydrates and related carbon compounds by bacteria is confusing on account of differences in techniques employed by different workers. For example, the nature of the basal medium employed in such studies is not indicated in many instances and the conflicting results obtained with the same organism by various workers are primarily due to the choice of different basal media (Smith, 1911; Burkholder, 1932; Lewis, 1930). Many of the plant pathogenic bacteria, particularly those of the Xanthomonas campestris group, produce ammonia from peptone in the basal medium, which neutralizes any acid produced from the carbohydrate under test. Ayers, Rupp and Johnson (1919) advocated use of a synthetic basal medium for studying the fermentation reactions of bacteria that produce acid and alkali simultaneously.

Some organisms, such as the plant pathogenic Corynebacteria, cannot, however, grow on an inorganic medium. Burkholder (1932) used a synthetic basal medium to study the carbon utilization by species of the X. campestris group in an attempt to separate species which could not be differentiated if a peptone base was used. Lewis (1930) using the same

technique more or less, studied the fermentation reactions of X. malvacearum and some other species of plant pathogens and obtained results that were conflicting with the ones found in literature (Smith, 1911).

In the present work, the fermentation of dextrose was first studied in a preliminary way by growing the organisms on nutrient dextrose broth in Durham fermentation tubes. Brom-thymol-blue was used as an acid-base indicator. The cultures were incubated at 25° C. and observations were recorded on alternate days.

None of the species under study produced any gas in two weeks. Xanthomonas campestris, X. lespedezae and Pseudomonas solanacearum produced an alkaline reaction which persisted. All the four species of Corynebacterium, Bacterium stewartii and Erwinia tracheiphila produced an acid reaction. Difficulty was encountered, however, in judging acid production by these species as these organisms are strongly aerobic and produce a copious yellow, slimy growth on the surface of the medium. The color of brom-thymol-blue in an acid medium is also yellow and hence the yellow growth of the organism interferes with the color change in the indicator.

To eliminate this difficulty, it was decided to use nutrient agar as the basal medium. Since none of the species produce any gas from dextrose, it was unnecessary to use fermentation tubes and a liquid medium. Nutrient agar containing

one per cent dextrose was prepared and made neutral to brom-thymol-blue, which was added to the medium at the rate of three ccs. of a 1.6 per cent alcoholic neutral solution per litre. This large amount of indicator was used in order to obtain sharp changes in color in the medium. Transfers on nutrient-dextrose-agar slants were made by streaking the surface of the slant and stabbing the butt, as is done in the case of Russell's double sugar agar for differentiation of organisms of the colon-typhoid group. No advantage was, however, secured by this method as none of the species grew anaerobically in the butt of the medium which showed no color changes in young cultures. In older cultures, the acid or alkali produced on the slope of the slant diffused in the body of the medium. Of the species under study, Bacterium stewartii in most cases produced acid from the carbohydrate, but utilized the same later. This was evidenced by the reversion of the slope of the slant to neutrality (green color) and finally to an alkaline reaction (blue color). The butt at the same time showed an acid reaction where the acid had not been utilized. This technique of using a solid medium with brom-thymol-blue as an indicator worked very well with the species of plant pathogens under study and is recommended for studying similar reactions of other plant pathogens which do not produce gas from carbohydrates. In cases where gas is produced, this technique cannot be used.

The results of these reactions with all the common carbohydrates are given in table 7. Since the basal medium used was nutrient broth, positive utilization of the fermentable substance was indicated by acid production. An alkaline reaction or no change in the medium indicated that either no acid was produced or if produced, it was neutralized by the ammonia resulting from the breakdown of the peptone. The results recorded in table 7 were taken at the end of 15 days growth of the organisms.

The results show that Xanthomonas campestris, X. lespedezae, and Pseudomonas solanacearum do not produce acid from any of the carbohydrates tested, when nutrient agar is used as the basal medium. As far as the latter two species are concerned, these results agree with those given for Pseudomonas solanacearum in Bergey's Manual (1939), and those of Ayers, Lefebvre and Johnson (1939) for Xanthomonas lespedezae. These workers used phenol red broth to study the fermentation reactions of the Lespedeza wilt pathogen which increased the pH of the medium from 7.2 to 7.6 - 7.9 in one month with glucose, lactose, sucrose, maltose, soluble starch and mannitol. As far as the descriptions of plant pathogens in Bergey's Manual (1939) are concerned, the basal medium used to study the fermentation reactions is not mentioned in any case. Xanthomonas campestris is said to produce acid but no gas from dextrose, sucrose, lactose, glycerol and mannitol. It is

Table 7. Production of acid from carbon compounds

Organism	No. of isolates	Arabinose	Xylose	Dextrose	Maltose	Cellobiose
Xanthomonas campestris	5	al	al	al	al	al
Xanthomonas lespedezae	3	al	al	al	al	al
Corynebacterium flaccumfaciens	5	ac	ac	ac	ac *	ac
Corynebacterium michiganensis	4	nc	nc	ac	ac *	ac
Corynebacterium sepedonicum	1	nc	nc	ac	nc	nc
Corynebacterium insidiosum	5	nc	nc	ac	ac	ac
Bacterium stewartii	5	ac *	ac *	ac *	ac *	ac *
Pseudomonas solanacearum	1	al	al	al	al	al
Erwinia tracheiphila	1	ac	ac	ac	ac	ac

Note: al = alkaline reaction; ac = acid reaction; nc = no chang

id from carbon compounds

- Xylose	Dextrose	Maltose	Cello-biose	Lac-tose	Sucrose	Melezi-tose	Pectin	Starch	I
al	al	al	al	al	al	al	al	al	
al	al	al	al	al	al	al	al	al	
ac	ac	ac *	ac	ac	ac	ac	ac	nc	
nc	ac	ac *	ac	ac	ac	ac	ac	nc	
nc	ac	nc	ac	ac	ac	ac	ac	nc	
nc	ac	ac	ac	ac	ac	ac	ac	nc	
ac *	ac *	ac *	ac *	ac *	ac *	ac *	ac *	ac *	
al	al	al	al	al	al	al	al	al	
ac	ac	ac	ac	nc	ac	nc	ac	nc	

lon; ac = acid reaction; nc = no change in reaction; * = reversion to neutra

ıc- ɔse	Sucrose	Melezi- tose	Pectin	Starch	Inulin	Escu- lin	Sali- cin	Gly- cerol	Dulci- tol	Man- nitol
.	al	al	al	al	al	sl	al	al	al	al
.	al	al	al	al	al	al	al	al	al	al
;	ac	ac	ac	nc	ac	ac	ac	ac	ac	ac
;	ac	ac	ac	nc	ac	ac	ac	ac	ac	ac
;	ac	ac	ac	nc	ac	ac	ac	al	al	al
;	ac	ac	ac	nc	ac	ac	ac	ac	ac	ac
;	ac *	ac *	ac *	ac *	ac *	ac *	ac *	ac *	ac *	ac *
.	al	al	al	al	al	al	al	al	al	al
;	ac	nc	ac	nc	nc	nc	nc	nc	nc	nc

in reaction; * = reversion to neutrality

assumed that nutrient broth was used as a basal medium. The present results with Xanthomonas campestris show that it does not produce acid from any of the carbohydrates tested. This discrepancy might be due to variations in strains as far as acid production from carbohydrates is concerned.

All the four species of Corynebacterium produced acid from most of the carbon compounds except the pentoses and starch. The only exception was C. sepedonicum which did not produce any acid from maltose and gave a distinct alkaline reaction with glycerol, mannitol and dulcitol.

Bacterium stewartii produced acid from all the substances tested and further used up the acid produced.

Erwinia tracheiphila produced acid from the pentoses, hexoses, disaccharides, trisaccharides and pectin but not from any of the other compounds.

Since Xanthomonas campestris, X. lespedezae, Pseudomonas solanacearum and Bacterium stewartii did not produce any acid from any of the carbon compounds in a peptone basal medium, it was thought necessary to use an inroganic basal medium to study the utilization of carbon compounds by these species. The medium used was a modification of Ayers, Rupp and Johnson's (1919) medium and consisted of:

$(NH_4)_2 HPO_4$	1.0 gm.
KCl	0.2 gm.
$MgSO_4 - 7H_2O$	0.2 gm.

$CaCl_2$	0.2 gm.
Agar	15.0 gm.
Water	1.0 litre
Carbon compound	10. gm.

The calcium chloride was dissolved separately and added to the bulk of the medium to prevent precipitation of calcium phosphate. Brom-thymol-blue was used as an indicator at the rate 3 ccs. of 1.6 per cent alcoholic solution per litre of the medium. In addition to all the carbon compounds used previously, formic, acetic, lactic, citric, tartaric, salicylic, oxalic and benzoic acids and ethyl alcohol were also tested. In the case of the organic acids, the reaction of the medium was readjusted with 0.1 N NaOH after addition of the acids. Ethyl alcohol was added to the sterile, cooled medium just prior to pouring plates. In every case, the various strains of the same species were inoculated in the same plate which was divided into quadrants. Transfers were made with a standard platinum loop, from young broth cultures. The plates were incubated at 25° C. and observations were made at the end of 5 and 15 days. Growth was recorded as negative only when it was not evident after 15 days. All isolates that grew were retested on the same medium.

As the only source of energy in every case was the particular carbon compound added to the basal medium, utilization of the carbon source was evidenced by growth of the

organisms. The change in the reaction of the medium accompanying growth was disregarded as an acid could result from the ammonium phosphate. The results are recorded in table 8.

The results show that the two species of Xanthomonas can utilize a large number of carbon compounds and are very much alike in this respect except that X. lespedezae cannot utilize mannitol. Of the organic acids tested, only acetic and citric can support growth of these two species.

Pseudomonas solanacearum on the other hand can utilize a very limited number of carbon sources. It differs from the Xanthomonas species in its inability to utilize xylose, maltose, lactose, melezitose, starch, esculin, and acetic acid but it can utilize glycerol.

Bacterium stewartii assumes an intermediate position between the two genera Xanthomonas and Pseudomonas in respect of its ability to utilize carbon compounds. It differs from both of them in that it utilizes arabinose, and does not utilize cellobiose and citric acid. It resembles the two Xanthomonas species on one hand in its ability to utilize maltose and resembles Pseudomonas solanacearum in its ability to utilize xylose, melezitose, starch and acetic acid. Further, it utilizes glycerol, as also can Pseudomonas solanacearum.

Table 8. Utilization of carbon compounds employing ammonium phosphate as source of nitrogen.

Organism	No. of isolates	Arabinose	Xylose	Dextrose	Maltose	Cellobiose	Lactose	Sucrose	Melezitose	Pectin	Starch
Xanthomonas campestris	5	-	+	+	+	+	+	+	+	+	+
Xanthomonas lespedezae	3	-	+	+	+	+	+	+	+	+	+
Bacterium stewartii	5	+	-	+	+	-	+	+	-	+	-
Pseudomonas solanacearum	3	-	-	+	-	+	-	+	-	-	-

of carbon compounds employing
phate as source of nitrogen.

-	-	+	+	Xylose
+	+	+	+	Dextrose
-	+	+	+	Maltose
+	-	+	+	Cellobiose
-	+	+	+	Lactose
+	+	+	+	Sucrose
-	-	+	+	Melezitose
-	+	+	+	Pectin
-	-	+	+	Starch
-	-	-	-	Inulin
-	-	+	+	Esculin
-	-	-	-	Salicin
+	+	-	-	Glycerol
-	+	-	+	Mannitol
-	-	-	-	Dulcitol
-	-	-	-	Ethyl alc.

-	-	-	-	Inulin	
-	-	+	+	Esculin	
-	-	-	-	Salicin	
+	+	-	-	Glycerol	
-	+	-	+	Mannitol	
-	-	-	-	Dulcitol	
-	-	-	-	Ethyl alc.	
-	-	-	-	Formic	Organic acids
-	-	+	+	Acetic	
-	-	-	-	Lactic	
+	-	+	+	Citric	
-	-	-	-	Tartaric	
-	-	-	-	Salicylic	
-	-	-	-	Oxalic	
-	-	-	-	Benzoic	

Utilization of Organic Nitrogen and its Relation to Pathogenicity

It has been pointed out previously that the species of Corynebacterium under study failed to grow in synthetic media containing potassium nitrate or asparagin as the source of nitrogen. These species evidently require a complex organic source of nitrogen. Very little work has yet been done on this phase of the plant pathogenic bacteria.

The results on reduction of nitrate show that only Pseudomonas solanacearum can reduce nitrates to nitrites while the rest of the species of wilt bacteria do not have this ability. From the species that do not reduce nitrates to nitrites, Xanthomonas campestris, X. lespedezae, and Bacterium stewartii can, however, grow on an inorganic nitrogen medium, but the Corynebacteria cannot.

Mushin (1938) studied the food requirements of Pseudomonas solanacearum and Corynebacterium michiganensis, both affecting tomato. She grew these two species on synthetic media containing different sources of carbon and nitrogen that are likely to be found in the xylem and phloem of host plants and found that asparagin, peptone, tyrosine and glutamic acid serve both as a source of carbon and nitrogen for Pseudomonas solanacearum. Corynebacterium michiganensis, on the other hand, could only grow on a medium containing peptone as

a source of carbon and nitrogen. Stapp (1930) found that only some proteins and amino acids can serve as a source of carbon and nitrogen for Corynebacterium michiganensis. The nitrogen requirements of the other wilt-bacteria have not been worked out.

In the present study the organisms were grown on a basal inorganic medium to which various organic nitrogen compounds (mainly amino acids) were added as a source of carbon and nitrogen. In one series, the nitrogen compound alone served as source of carbon and nitrogen, and in another series of cultures, one per cent dextrose was added as a source of carbon. The basal medium was the same as that used by Mushin (1938) and consisted of:

K_2HPO_4	3.1 gm.
KH_2PO_4	0.8 gm.
KCl	0.2 gm.
$MgSO_4$	0.2 gm.
Water	1 litre

In the preliminary experiments, the medium was used as a solid medium by addition of 1.5 per cent washed, purified agar. The pH of the medium was adjusted to neutrality to brom-thymol-blue, which was incorporated in the medium at the rate of 1 cc. of a 1.6 per cent alcoholic solution to a litre. The amino acids used were glycine, β-alanine, l-leucine, l-tyrosine, l-tryptophane, l-cystine, d-lysine,

d-arginine, l-aspartic acid, d-glutamic acid, creatine, creatinine, and d-l-isoleucine. In addition to these amino acids, choline, paraamino benzoic acid and sarcosine were also tested. In each case 0.2 per cent of the test substance was used, except tryptophane and arginine, where only 0.1 per cent was used. Protease peptone constituted the check. The reaction of the medium was lowered in some cases after the amino acids were added and was readjusted with 0.1 N NaOH. Cystine was dissolved in dilute hydrochloric acid before addition to the medium.

Plates were poured in each case and divided into eight sections on the bottom with a glass marking pencil. Each sector was inoculated with one of the eight organisms under study; Erwinia tracheiphila was not included. Broth cultures were used for inoculations with a straight needle. The isolates used were of proved pathogenicity. The plates were incubated at 25° C. and observations were made at the end of a week.

Whenever growth was obtained, the results were confirmed by reinoculation in tubes of a liquid medium of the same composition, using a serial transfer technique. A tube of the medium under test was lightly inoculated and incubated for a week. At the end of this period, this tube was used to inoculate another tube of the same medium and an agar slant. Growth was recorded as positive if the organism

Table 9. Utilization of organic nitrogen with and without dextrose as source of carbon.

Source of nitrogen	*Xanthomonas campestris*		*Xanthomonas lespedezae*		*Pseudomonas solanacearum*		*Bacterium stewartii*	
	with dextrose	without dex.	with dextrose	without dex.	with dextrose	without dex.	with dextrose	without dex.
Glycine	-	-	+	-	+	-	+	-
β-alanine	-	-	-	-	+	+	+	-
Leucine	+	-	+	-	+	-	+	-
Isoleucine	+	-	+	-	-	-	+	-
Tyrosine	+	-	+	-	+	+	+	-
Tryptophane	+	-	+	-	+	-	+	-
Cystine	+	+	+	-	+	-	+	-
Lysine	+	+	+	-	+	-	+	-
Arginine	-	-	-	-	+	-	+	-
Aspartic acid	+	+	+	-	+	+	+	-
Glutamic acid	+	+	+	+	+	+	+	+
Creatine	+	-	+	-	+	-	-	-
Creatinine	+	-	+	-	+	-	+	-
Choline	+	-	+	-	-	-	+	-
Para-amino-benzoic acid	-	-	-	-	-	-	+	-
Sarcosine	-	-	+	-	-	-	+	-
Proteose peptone (check)	+	+	+	+	+	+	+	+

rium artii without dex.	Corynebacterium flaccumfaciens with dextrose	Corynebacterium flaccumfaciens without dextrose	Corynebacterium michiganensis with dextrose	Corynebacterium michiganensis without dextrose	Corynebacterium insidiosum with dextrose	Corynebacterium insidiosum without dextrose	Corynebacterium sepedonicum with dextrose	Corynebacterium sepedonicum without dextrose
-	-	-	-	-	-	-	+	-
-	-	-	-	-	-	-	-	-
-	+	-	+	-	-	-	-	-
-	-	-	-	-	-	-	-	-
-	+	-	+	-	-	-	+	-
-	-	-	-	-	-	-	-	-
-	+	-	+	-	-	-	-	-
-	+	-	+	-	-	-	+	-
-	+	-	+	-	-	-	-	-
-	+	-	+	-	-	-	-	-
+	+	-	+	-	+	-	+	-
-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	+	-
-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-
+	+	+	+	+	+	+	+	+

could be cultivated on the third successive agar slant. The results are given in table 9.

Of all the organisms under study, Bacterium stewartii made the most profuse growth on media containing dextrose. The two species on Xanthomonas also grew well on these media but not as well as the Stewart's wilt organism.

The results show that the species of Corynebacterium are very inactive in utilization of organic nitrogen; C. insidiosum was the most inactive in this group of organisms. None of these organisms could utilize the substances tested as sole source of carbon and nitrogen.

Of all the substances, glutamic acid, in the presence of dextrose, supported growth of all the species. Without any addition of dextrose, it supported growth of the Xanthomonas species, Pseudomonas solanacearum, and Bacterium stewartii. Some striking differences were shown by the species of Xanthomonas; X. campestris could utilize cystine, lysine and aspartic acid as sole sources of carbon and nitrogen while X. lespedezae could not.

Pseudomonas solanacearum could utilize β- alanine, tyrosine, and aspartic as sole sources of carbon and nitrogen.

These results may have a positive correlation with the pathogenesis and host range of the organisms.

When this work was being carried out, some tomato plants inoculated with different strains of Corynebacterium michiganen-

sis were available. Of the strains under test, two strains showed considerable differences in their virulence. One of the strains, CM-6, was very weakly virulent and produced only a few necrotic cankers on the plants, which never wilted. The other strain, 7433, was quite virulent and produced characteristic leaf symptoms on inoculated plants, 75 per cent of which wilted in one month. Since these two strains showed differences in virulence a study of their nitrogen utilization was undertaken. Isolations were made from diseased plants and ten single colony cultures were made from a strain CM-6 and four from strain 7433.

All these 14 strains were identical in their morphological and physiological characters. All were Gram positive rods, yellow colored, produced hydrogen sulphide, and coagulated milk without peptonization. Further, all produced acid from common carbohydrates.

In order to find out whether these strains differed in their ability to utilize organic nitrogen, they were grown on a synthetic medium to which were added organic nitrogen compounds with and without dextrose. The basal medium and nitrogen compounds were the same as used previously and the technique was essentially the same.

On the media containing the organic nitrogen compounds as the sole source of carbon and nitrogen, the 10 isolates of strain CM-6 did not make any growth. The four isolates

of strain 7433, on the other hand grew in one week on media containing aspartic acid and glutamic acid as sole sources of carbon and nitrogen. These results were confirmed on liquid media of the same composition by the serial transfer technique.

In another series, the isolates were grown on media containing the same nitrogen sources as before but one per cent dextrose was added as a source of carbon to the basal medium. The results of this test are recorded in table 10.

In order to find out whether the various isolates differed in their pathogenicity to tomato plants, inoculations were made on young tomato seedlings. Two series of inoculations were made. In one series, seedlings about two weeks old were inoculated by pricking the cotyledons and the stem with a flamed needle carrying a small amount of bacterial culture. Four plants were inoculated with each isolate. Similar seedlings pricked with a sterile needle provided a check. The pots containing the tomato seedlings were kept on a greenhouse bench where the mean air temperature was about 85° - 90° F.

One week after inoculation, plants inoculated with isolates 11, 12, 13 and 14 showed typical symptoms of the disease. Most of the inoculated plants showed drooping and wilting of leaves and white, cankerous areas on the stems around points of inoculation, which extended above and below

Table 10. Utilization of organic nitrogen with dextrose as source of carbon by isolates of *Corynebacterium michiganensis*.

Source of nitrogen	Strain CM-6	Isolates 1	4	5	6	7	8	9	10	Strain 7433	Isolates 11	12	13	14
Glycine	-	-	-	-	-	-	-	-	-	-	+	-	+	+
β-alanine	-	+	-	-	-	-	-	-	-	-	+	+	+	+
Leucine	-	+	-	+	-	-	-	-	+	+	+	-	-	+
Isolencine	-	-	-	-	-	-	-	-	-		+	+	+	+
Tyrosine	-	-	-	-	-	-	-	-	-	+	+	+	+	-
Tryptophane	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Cystine	-	-	-	-	-	+	-	-	-	+	+	+	+	+
Lysine	-	-	-	-	-	-	-	-	-	+	+	+	+	-
Arginine	-	-	-	-	-	-	-	-	-	+	-	-	-	-
Aspartic acid	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Glutamic acid	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Creatine	-	-	-	-	-	-	-	-	-	-	+	+	+	-
Creatinine	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Choline	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Para-amino-benzoic acid	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Sarcosine	-	-	-	-	-	-	-	-	-	-	-	+	+	+
Proteose peptone (Check)	+	+	+	+	+	+	+	+	+	+	+	+	+	+

the sites of inoculations. In one month, 2-3 plants in each pot had wilted. At the same time, plants inoculated with the isolates of CM-6 showed only a little stunting but no wilt.

In another series, seedlings about three weeks old were inoculated with each of the isolates and also with the parent strains. Inoculations were made by cutting off the tops of the seedlings with a flamed scalpel and applying inoculum to the cut end, as recommended by Ark (1944). Adequate checks consisted of plants cut in a similar manner but uninoculated with the bacteria.

In four days after inoculations were made, a majority of the plants inoculated with isolates 11, 12, 13 and 14 showed characteristic wilting of leaves, which started at the tips. None of the other isolates produced any symptoms on inoculated plants. After one month, 50 to 70 per cent of the plants inoculated with isolates 11, 12, 13 and 14 had wilted whereas those in the other series showed only stunting but no deaths.

These results indicate a correlation between the virulence and ability to utilize amino acids as source of nitrogen and/or carbon as far as Corynebacterium michiganensis is concerned. This species is known to be extremely variable; yellow, pink and white strains have been described (Bryan, 1931) and color is shown to be associated with virulence. Ark (1946) obtained a white mutant of Corynebacterium michiganensis by treating the yellow parent strain with acenapthene. The mutant was

more virulent than the parent strain but otherwise like it in morphology and physiology. In the light of results of the present, it seems that the mutant might be expected to behave differently in respect to amino acid utilization.

Bacterium stewartii is another wilt pathogen which shows variations in virulence and it would be worthwhile to find out whether these variations are associated with amino acid utilization. McNew (1938) made a start in this direction but used only nitrates. He found virulent cultures of Bacterium stewartii that could reduce nitrates and he was also able to show that when slightly virulent cultures were restored to virulence by host passage, they had acquired the ability to utilize inorganic nitrogen.

It is safe to speculate that similar differences would be found in the utilization of amino acids. This would provide a new field of research in the case of phytopathogenic bacteria and may shed light on host specificity and host range.

NAMES FOR THE WILT BACTERIA

As had already been said, the wilt bacteria are a taxonomically heterogeneous group of organisms. According to the system of classification used in Bergey's Manual, 6th edition, these bacteria represent five genera in four families of the Eubacteriales, namely Enterobacteriaceae (Erwinia tracheiphila), Pseudomonadaceae (Pseudomonas solanacearum and the two species of Xanthomonas), Corynebacteriaceae (four species of Corynebacterium), and Bacteriaceae (Bacterium stewartii). Of the four families, Corynebacteriaceae is well represented by the wilt bacteria (four out of nine species), and some new information regarding this genus (as now constructed) is available as a result of the present study. As far as the five genera are concerned, the present work has brought out some new information and confirmed some characters which are of diagnostic value for these five genera, but since only a very few species of each genus were studied, no generalizations can be made.

The results of the present work show that the wilt-producing species of Corynebacterium are totally different in their physiology from members of the other genera Pseudomonas, Xanthomonas, Bacterium, and Erwinia. The Corynebacteria are very selective and evidently require complex sources of

nitrogen. The results of experiments with the amino acids show that none of these compounds tested can singly support the growth of any of the species but some of the pathogens can utilize a few as sources of nitrogen if dextrose is supplied as a source of carbon. Further, the results on strains of C. michiganensis varying in virulence have shown that nitrogen metabolism may have a bearing on parasitism. The same can possibly be true of the other wilt producing Corynebacteria. All the four species were, however, able to grow on peptone as the sole source of carbon and nitrogen and it can therefore be assumed that these organisms require some growth promoting substances such as vitamins in addition to pure amino acids. It would be worthwhile to find out whether different combinations of amino acids as sole source of carbon and nitrogen would be able to support growth of these selective species.

The wilt producing members of the genera Pseudomonas, Xanthomonas and Bacterium show very close affinities regarding their physiology. All can utilize inorganic nitrogen in the form of nitrate or ammonia and can also use glutamic acid as sole source of carbon and nitrogen.

The genus Corynebacterium as defined in the 6th edition of Bergey's Manual contains both motile and non-motile forms. The original Corynebacterium of Lehmann and Neumann, 1896, was based to include only non-motile forms. Conn and Dimmick (1947)

have severely criticized the inclusion of C. flaccumfaciens in that genus, as it is motile. It would appear that these authors put more stress on morphology as a character for demarcation of genera and do not consider the close physiological affinities of organisms slightly different in morphology. They are of the opinion that the inclusion of C. michiganensis in the genus is perhaps justified since this organism is non-motile, gram positive, shows tendency to branching, and does not liquefy gelatin. It is of interest to note that in the present work, three of the four cultures of this species, including the type culture, did liquefy gelatin both in a nutrient gelatin medium and on Frazier's gelatin. Corynebacterium flaccumfaciens has the same characters except that it is motile. It further has the same nitrogen metabolism as the rest of the wilt-producing species of Corynebacterium. It is felt that there is justification in including C. flaccumfaciens in that genus. Further, it is highly doubtful whether flagella are the true means of locomotion or are merely appendages of the bacterial cell. Pijper (1947) studied the motility of several species by using methylcellulose and came to the conclusion that motility of bacteria is not dependent on the so called "flagella" but is a function of the bacterial cell itself. He also showed that very motile bacteria need not exhibit any "flagella". Their development depends upon the production of a good slime layer and he suggested the

term "polysaccharide twirls" to replace the term "flagella". In the light of these results, the terms "motile" and"non-motile", based on the presence or absence of flagella, lose their significance. Conn and Dimmick (1947) seem to have overlooked this.

The only species of wilt-bacteria that has no definite taxonomic position at present is the Stewart's wilt pathogen, Bacterium stewartii. E. F. Smith (1905) placed it in his new genus Aplanobacter along with some other non-motile species and cited the anthrax bacillus (a sporeformer) as the type of the genus. He stated that "for the present non-sporiferous forms resembling Aplanobacter anthracis are also included in this genus, but if it shall be decided, later on, that the difference between sporiferous and non-sporiferous forms is of generic significance, then the latter may be excluded." Since the name Bacillus was later reserved for the sporeformers, Smith's Aplanobacter naturally became a synonym. Moreover, according to his own statement, the non-sporulating plant pathogens would have been excluded fro Aplanobacter even if that genus had become established. Bergey, et al (1939) put the Stewart's wilt organism in their Phytomonas, in Appendix II to that genus along with the gallformers and other wilt producers. Dowson (1939) placed it in his new genus Xanthomonas but later excluded in from it (Dowson, 1943). Starr and Weiss (1943) found that it could grow on their

synthetic asparagin medium, while species of Xanthomonas could not. Starr and Burkholder (1942) found that it was not lypolytic while species of Xanthomonas were. They recommended that the name Phytomonas stewartii be temporarily retained for it. In the new 6th edition of Bergey's Manual, it appears as Bacterium stewartii following the recommendation of Breed and Conn (1936) "that Bacterium be accepted as a temporary generic term with an admittedly unrecognizable type species, Bacterium triloculare Ehrenberg, to include those species of nonsporeforming, rod-shaped, motile or nonmotile bacteria whose relationships to other bacteria are not clear".

The morphological and physiological characters of Bacterium stewartii clearly show that it is related to Xanthomonas on one hand and Pseudomonas on the other. It resembles Xanthomonas in its yellow color and gum-production, and resembles Pseudomonas by its ability to utilize asparagin as sole source of carbon and nitrogen and lipolytic activity. It resembles members of both the genera in that it can utilize inorganic nitrogen. It is thus evident that Bacterium stewartii belongs in the Pseudomonadaceae. It differs from Pseudomonas and Xanthomonas more than it resembles them. Disregarding the character of motility for the present, it differs from Xanthomonas species in that it does not liquefy gelatin, does not hydrolyze starch, and does not peptonize milk. It also differs from species of Pseudomonas by its inability to produce

a green-fluorescent pigment, and non-production of alkali in litmus milk. Its affinities to Pseudomonas are more marked than to Xanthomonas.

Utilization of carbon sources reveals still more differences. Bacterium stewartii produces acid from most sugars in a peptone basal medium. In an inorganic basal medium, it can utilize arabinose whereas the species of Xanthomonas and Pseudomonas studied cannot; it is further characterized by its inability to utilize cellobiose and citric acid. These characters are, however, of secondary importance.

As regards utilization of amino acids as source of nitrogen in presence of dextrose, Bacterium stewartii can utilize a very large number of these substances. It was the only species that could utilize para-amino-benzoic acid and sarcosine.

Burkholder (1930) suggested that most of the yellow, motile, plant pathogens (now in Xanthomonas) be placed in the genus Flavobacterium in the Bacteriaceae (now in the Achromobacteriaceae; Bergey's Manual, 6th ed.). This genus is a heterogeneous mass of gram positive and gram negative, motile and nonmotile, yellow to orange, soil and water bacteria. The chief generic character is "feeble powers of attacking carbohydrates, occasionally forming acid from hexoses, but no gas". This genus, in the 5th edition of Bergey's Manual, contains 58 species divided into three groups on motility. Twenty-three species are motile,

nine are motile but the location of flagella is not recorded, and the remaining 26 are non-motile. An examination of the descriptions shows that most of them are very incomplete. Of the 26 non-motile species, four (including one gram positive one) are reported as producing acid from dextrose and one producing acid and gas. Most of them liquefy gelatin but do not form indole. Litmus milk reactions are variable. If the Stewart's wilt organism has to be included in this genus, then the genus will have to be emended since its character is the feeble powers of attacking carbohydrates. It is interesting to note that this genus has now been transferred to a new family, Achromobacteriaceae, with Achromobacter, a non-pigmented genus as the type. Bacteriaceae now contains only one genus, Bacterium, with six sub-genera.

It is felt that the Stewart's wilt organism deserves to be placed in a new genus in the Pseudomonadaceae. However, a large number of isolates will have to be examined before definite recommendations are made. The Stewart's wilt organism is a variable species as regards virulence and cultural characters (Ivanoff, et al, 1938) and any future work should be directed towards a study of all the variants that could be had. The present study dealt with only five isolates and the author is not prepared to make any definite recommendations regarding the taxonomic position of this organism on the evidence at his disposal.

HOST RELATIONS OF THE WILT BACTERIA

An attempt has been made in the following table to summarize information regarding the pathogenesis of the organisms under study; this information has been compiled from the existing literature on the subject.

Table 11. Comparative pathogenesis of nine bacterial wilt organisms.

Organism	Common host	Mode of host invasion	Host tissue invaded	Mode of perpetuation
Xanthomonas campestris	crucifers	hydathodes, wounds, stomata-?	xylem	on seed; in soil
Xanthomonas lespedezae	annual lespedezas	wounds, stomata	xylem	in or on seed
Corynebacterium flaccumfaciens	common bean	wounds	xylem	in seed
Corynebacterium michiganensis	tomato	wounds; stomata	phloem	on seed
Corynebacterium sepedonicum	potato	wounds, roottips	xylem	in seed tubers
Corynebacterium insidiosum	alfalfa	wounds	xylem	on seed; in soil
Bacterium stewartii	sweet corn	wounds, hydathodes	xylem	on seed; in soil; in insects
Pseudomonas solanacearum	members of Solanaceae	wounds	xylem	in soil
Erwinia tracheiphila	cucumber	wounds	xylem	in cucumber beetles

Invasion through Wounds

An examination of table 11 shows that the most common method of host invasion is through wounds, although some of the pathogens are able to invade the host through natural openings such as hydathodes and stomata.

The type of wounds is diverse. They may be caused naturally during the growth of the plant as when the cotyledons unfold or new roots arise. Some species of wilt bacteria like Corynebacterium michiganensis and C. flaccumfaciens, which are seed-borne, gain entrance to their hosts through wounds on the cotyledons or young leaves. Root affecting insects cause injuries on the roots of members of the Solanaceae and sweet corn, through which Pseudomonas solanacearum and Bacterium stewartii gain entrance respectively. The cucumber beetle, which feeds on leaves, is responsible for transmitting Erwinia tracheiphila. Wounds caused during transplanting and "topping" tomatoes provide a means of entry for Corynebacterium michiganensis.

Of the nine species of wilt-bacteria under study, three pathogens, namely Corynebacterium flaccumfaciens, C. insidiosum and Pseudomonas solanacearum have not been shown yet to invade their host in any other manner than through wounds. Of these three, the two species of Corynebacterium are carried on the seed and invade the young seedlings through wounds on

the cotyledons or on roots. Pseudomonas solanacearum seems to be the only species that persists mainly in the soil, and invades its hosts through wounds on roots.

Invasion through Stomata and Hydathodes

Of the nine species of wilt bacteria under study, the only pathogen that normally invades the host through natural openings is Xanthomonas campestris. This organism enters the cabbage plant through hydathodes that are situated on the leaf margins at the termination of veins. Smith (1911) records invasion of maize by Bacterium stewartii through hydathodes but this is not the normal phenomenon in Stewart's wilt.

Stomatal invasion has been reported in the case of Xanthomonas campestris, X. lespedezae, and Corynebacterium michiganensis. Drechsler (1919) obtained stomatal invasion of cotyledons of cabbage seedlings by the "black-rot" pathogen but true leaves could not be infected in a like manner. Ayers, Lefebvre and Johnson (1939) obtained stomatal infection of lespedeza seedlings by Xanthomonas lespedezae but only when the seedlings were very young with only the primary leaves exposed. Bryan (1930) reported leaf infection on tomato by Corynebacterium michiganensis but the resulting lesions remained very inconspicuous and did not enlarge. In her

experiments, infection occasionally reached the vascular system from infected areas lying over the very large bundles of vessels on the extreme leaf margins.

Infection of potato by Corynebacterium sepedonicum through unwounded root tips has recently been reported by Tyner (1946) in greenhouse experiments. However, he employed large amounts of inocula, not comparable to conditions prevailing in nature and this type of infection, according to him, does not normally occur in the field.

It seems therefore that Xanthomonas campestris is the only wilt pathogen that is able to invade the host and spread in it as a result of entrance through natural openings, hydathodes in this case, but not through stomata. The lespedeza wilt organism can invade its host through stomata but in very young seedlings only. It was thought worthwhile, therefore, to find out why stomatal invasion is not a common phenomenon in cabbage and lespedeza wilts.

The reasons why stomatal invasion cannot occur have not been investigated but one of the factors could be the nature of the leaf surface, especially in cabbage. Cabbage leaves are covered with a thick coating of wax and do not allow the formation of a film of water on the leaf surface, which is essential for bacterial invasion through stomata. Anderson and Henry (1946), working with Piricularia oryzae, found that use of sodium oleate as a surface tension depressant and gelatin as an adhesive incorporated in a water suspension of

spores of the fungus gave higher amounts of leaf infection on rice than a suspension made in plain water alone. The most effective combination of the chemicals for Piricularia oryzae was 0.05 per cent soldium oleate and 0.25 per cent gelatin.

The same technique was used in the present experiments. However, it was necessary to determine the limiting concentrations of the chemicals which would not be toxic to the bacteria and at the same time be high enough to be effective on cabbage and lespedeza leaves.

One per cent stock solutions of sodium oleate and gelatin were therefore prepared and dilutions ranging from 0.01 to 0.05 per cent sodium oleate in 0.25 gelatin in water were made. Test tubes were filled with each of the dilutions, sterilized and inoculated lightly with Xanthomonas campestris and X. lespedezae. Duplicate tubes were inoculated in each case. Distilled water served as check. The viability of each organism in the various concentrations of sodium oleate was determined by making transfers to agar slants at the end of 12, 36, and 72 hours. The tubes were vigorously shaken before making transfers, to distribute the organisms evenly in the liquid. Whenever growth occurred on the agar slants, it was taken as proof of viability of the bacteria in the particular solution at the end of a particular period of incubation.

Table 12 shows the results of this experiment.

Table 12. Effect of concentrations of sodium oleate on viability.

Composition of test solution	Viability after hours					
	12		36		72	
	X. campestris	X. lespedezae	X. campestris	X. lespedezae	X. campestris	X. lespedezae
0.25% gelatin + 0.01% sod. ole.	+	+	+	+	+	+
0.25% gelatin + 0.02% sod. ole.	+	+	+	+	+	+
0.25% gelatin + 0.03% sod. ole.	+	+	+	+	+	+
0.25% gelatin + 0.04% sod. ole.	+	+	+	+	+	+
0.25% gelatin + 0.05% sod. ole.	+	+	+	+	+	+
0.25% gelatin alone	+	+	+	+	+	+
Water alone	+	+	+	+	+	+

The results show that both the species can withstand a concentration of 0.05 per cent sodium oleate in 0.25 per cent gelatin for 72 hours. In order to confirm these results, nutrient-dextrose-broth containing 0.05 per cent sodium oleate was prepared and tubes of this medium were inoculated with Xanthomonas campestris and X. lespedezae. Both the species

grew on the medium as shown by the turbidity produced.

Young cabbage seedlings, with four leaves each, and lespedeza seedlings about three weeks old were next inoculated by spraying suspensions of Xanthomonas campestris and X. lespedezae respectively, made up in sterile water containing 0.25 per cent gelatin and 0.01, 0.03, and 0.05 per cent sodium oleate. The plants were incubated in a moist chamber for 72 hours before and after inoculation. Eight plants of cabbage and twenty of lespedeza were inoculated with each treatment. Checks were inoculated with a water suspension of bacteria. The plants were exposed to invasion by bacteria by spraying the suspension, in every case, with an atomizer and both sides of the leaves were inoculated. It was observed in the course of inoculations that the suspensions containing sodium oleate 0.03 and 0.05 per cent spread evenly on the leaf surface both in cabbage and lespedeza. Water suspensions, on the other hand, accumulated in drops on the leaves and ran off. It was further observed that the cotyledons in both cabbage and lespedeza were considerably less waxy than the leaves and even a water suspension formed very good films on them. This might be responsible for the fact that cotyledons can be readily infected by spraying a suspension of bacteria on them (Drechsler, 1919).

Five days after the plants were sprayed with bacterial suspensions, two plants of cabbage where 0.03 per cent sodium

oleate was used showed symptoms of stomatal infection. This was evidenced by the "leaf-spot" type of lesions occurring on leaves away from the margins. In hydathode infection, lesions start along the margins of leaves where the bacteria gain entrance. All plants showed marginal infection in ten days. As the necrotic spots enlarged somewhat in size, they coalesced with the lesions originating from the margins of the leaves and two weeks after inoculation, large portions of leaves were involved.

In lespedeza, seven of the 20 inoculated plants showed necrotic spots on leaves in six days and in two weeks all these plants had wilted. It was also noticed that the cotyledons of most of the plants got infected and dropped off. Infection was not, however, evident on the leaves except in the seven plants which showed it.

Isolations were made from the necrotic lesions on leaves in both cabbage and lespedeza and the causal agents were readily isolated.

The experiment was repeated with cabbage using only one strength of sodium oleate, 0.03 per cent, since this concentration gave the best result. Plants of three different ages were used: two weeks, one month and two months. Eight plants were inoculated in each case. The various treatments used were water alone, 0.03 per cent sodium oleate alone, water suspension of Xanthomonas campestris and a suspension made in

0.03 per cent sodium oleate. Inoculations were made with an atomizer and the plants were incubated in a moist chamber for 72 hours before and after inoculations.

A week after the plants were exposed to infection, numerous small, yellow lesions appeared on leaves of many of the plants sprayed with a suspension of the bacteria made in 0.03 per cent sodium oleate. At the same time, marginal infection of the leaves through the hydathodes was also noticed. The lesions were less on leaves of younger plants than on those of older ones but they enlarged somewhat and coalesced with the lesions rapidly spreading inwards from the margins of the leaves. On older leaves, of one and two month old plants, infection spots remained very small, about 1-2 mm. in diameter and contained dead, brown areas at their centers. On these plants, hydathode infection was as severe as on younger plants and spread rapidly over the whole leaf.

Plants inoculated with a water suspension of bacteria also developed some hydathode infection in every case and the development of this infection was as rapid as in the case of plants on which sodium oleate was used.

Plants sprayed with water alone and with a 0.03 per cent solution of sodium oleate did not show any infection or injury by sodium oleate.

These results indicate that stomatal infection is possible in cabbage and in lespedeza but that the pathogen cannot reach

the vascular elements of the plant in this manner. This may be due to the inability of the organism to grow in intercellular spaces in the leaf just below the stomata. Moreover, the bacteria cannot get a direct entrance to the vascular system through the stomata, while this is possible in the case of infection through hydathodes which are situated at the termination of veins.

The characteristic symptom produced on the host by members of the genus Xanthomonas is "leaf-spot" and "blight"; X. campestris and X. lespedezae are the only wilt producers in this genus. Other species such as X. phaseoli are able to invade the vascular system of the host plant but the primary symptom is "blight". All the members of the X. campestris group are very much alike in physiology and cultural characters and can be differentiated only on basis of their pathogenicity to particular hosts. Why is it then that the cabbage and lespedeza pathogens should be restricted to the vascular system of their hosts? One of the factors may be the inability of the wilt producers to attack cell walls in the leaf tissue. These organisms have very simple nutritional requirements and it seems doubtful that their inability to grow in the intercellular spaces is due to lack of proper nutrition. The only feasible possibility seems to be the inability of the wilt-bacteria to elaborate either an enzyme or one or more chemicals that would break down the cell wall. The ability of the "soft-

rot" organisms to elaborate protopectinase has been proved (Waldee, 1945), and the characteristic symptom produced by these bacteria is due to the enzyme. The "leaf-spot" and "blight" organisms of the genus Xanthomonas might have the ability to produce some similar enzyme or chemical, which is not possessed by the wilt bacteria.

Host Tissue Invaded

The wilt bacteria in all cases cause a vascular necrosis of the host primarily, which results in the wilting of the host plant. Xylem is invaded in all cases except by the tomato canker organism, Corynebacterium michiganensis which invades the phloem and also comes out on the surface of the host causing cankerous patches on the stem. Pseudomonas solanacearum is another example of parenchymo-vascular organism. In the case of lespedeza wilt again, the pathogen comes out on the surface of blighted leaves and through cracks in stems of plants harboring systemic vascular infection. The rest of the wilt bacteria, Xanthomonas campestris, Corynebacterium flaccumfaciens, C. sepedonicum, C. insidiosum, Erwinia tracheiphila and Bacterium stewartii strictly invade the xylem and never come out on the host surface. In most cases the parenchyma surrounding the xylem vessels is destroyed after death of the vessels, but whether

this results through the escape of bacteria through the pits on the vessels or due to the disintegration of the walls of the vessels, has never been proved. In any case, the bacteria are never occluded in the parenchyma before the vessels are completely filled by them.

The process of wilting as a result of systemic invasion by the bacteria may be sudden or gradual. Sudden wilting of infected plants is characteristic of Erwinia tracheiphila on cucumbers and Pseudomonas solanacearum on Solanaceae. Diseased plants in these cases may collapse suddenly without showing any outward symptom. Bacterium stewartii also causes sudden wilting sometimes when young seedlings are affected. Leaf symptoms are exhibited by many of the species of wilt bacteria. These symptoms take the form of yellowing and curling of leaves (species of Corynebacterium), and eventual drying up, blight (Xanthomonas lespedezae), or blackening of veins accompanied by yellowing and drying (X. campestris). Leaf stripe is exhibited by Bacterium stewartii, and is a characteristic symptom of Stewart's wilt in the early stages of the disease.

The movement of wilt-bacteria inside their host varies with the different organisms. In the case of Xanthomonas campestris and X. lespedezae, the bacteria gain entrance usually through the leaves and after producing symptoms there, move downwards through xylem in the stem. Bacteria have not

been found in the roots in Lespedeza wilt and this is probably due to the death of the plant when the aerial parts are killed. This is not the case in cabbage, where wilting is more gradual.

In the case of Stewart's wilt, the movement of the organism inside the plant is upwards in the early stages of the disease. This is shown by the appearance of symptoms in leaves above the point of inoculation on the stem in greenhouse tests. In the later stages of the disease, when the aerial parts of the plant are killed, the organism is also found in the roots of the plant. Root infestation is, of course, evident when infection in the field occurs through the roots. In the case of Pseudomonas solanacearum, infection through roots is the rule. The bacteria in this case travel rapidly upward in the plant and cause severe wilting. In artificial inoculations, the organism can travel both upward and downward inside the plant. This pathogen can also cause epinasty of the petioles, and adventitious root development (Grieve, 1936). The movement of Erwinia tracheiphila inside the host plant is similar to that of Ps. solanacearum. The organism is always inoculated into the aerial parts by the bites of cucumber beetles and when once inside, the bacteria rapidly travel both upwards and downwards in the xylem.

The Corynebacteria form a group of wilt-bacteria by themselves. As far as is known, movement of these organisms is generally upwards except in the case of Corynebacterium

michiganensis. Ark (1944) showed that the best method of obtaining artificial infection on tomato plants with C. michiganensis was cutting off the tops of the plants with a knife laden with bacteria. This method gave higher and more severe infection than when the plants were inoculated by means of a hypodermic syringe.

Since the organism invades the phloem, it would seem natural that direct inoculation of the phloem by bacteria (as done by cutting off the top of the plant with a contaminated knife) would give severe infection. Another characteristic of this organism is partial wilting of infected plants. The disease sometimes occurs only on one side of the stem whereas the other side remains healthy. Bryan (1930) reported that roots of tomatoes are sometimes invaded by this organism.

The movement of bacteria inside the host is always upwards in the case of C. sepedonicum and C. insidiosum, both of which infect the host through the roots, and cut stems. Infection of roots is never noticed in bean wilt caused by C. flaccumfaciens. Primary infection in this case takes place through the germinating seed. The organism then follows an upward path in the xylem, finally entering the seed.

Host Range

Review of literature.

The natural hosts of the wilt-bacteria are mostly annuals and comprise members of the families Cruciferae (Xanthomonas campestris), Leguminosae (X. lespedezae, Corynebacterium flaccumfaciens and C. insidiosum), Solanaceae (C. michiganensis, C. sepedonicum, Pseudomonas solanacearum), Gramineae (Bacterium stewartii), and Cucurbitaceae (Erwinia tracheiphila). An attempt is made below to review the existing literature on the host range of the wilt-bacteria.

Xanthomonas campestris. This species was first reported by Pammel (1895) on rutabaga. Elliott (1930) lists the following hosts of this organism: Brassica arvensis, B. campestris, B. chinensis, B. napus, B. nigra, B. oleracea acephala, B. oleracea botrytis, B. oleracea capitata, B. oleracea caulo-rapa, B. pekinensis, B. rapa, Matthiola incana, and Raphanus sativus. The susceptibility of stocks (Matthiola incana) to the "black-rot" organism has been questionable. It appears that this organism is restricted to the Crucifers.

Xanthomonas lespedezae. Ayers, Lefebvre and Johnson (1939) described this species on annual lespedezas (Lespedeza stipulacea and L. striata) in Virginia. Bacterial wilt was also observed on perennial species of Lespedeza in the field, but

inoculations in the greenhouse showed that Lespedeza capitata, L. daurica, L. frutescens, L. inochanica, L. procumbens, L. sericea and L. virginica were susceptible. L. daurica (90355,a prostrate form), L. bicolor, L. cyrtobotrya, L. formosa, L. hirta, L. latissima, and L. thunbergii appeared to be highly resistant. The organism was also able to produce small necrotic areas on leaves of Melilotus alba inoculated by means of needle pricks, but no symptoms were produced by the organism when inoculations were made on alfalfa, red clover, white sweet clover, ladino clover, broad bean, two species of Crotolaria, refugee bean, kudzu, lotus, and eight varieties of soy bean. It would seem that this organism is restricted to the genus Lespedeza only.

Corynebacterium flaccumfaciens. Hedges (1922) first described this organism on common beans (Phaseolus vulgaris) Elliott (1930) records P. vulgaris and P. lunatus macrocarpus as the hosts of this pathogen, and soybeans were successfully inoculated artificially. Burkholder (1930) obtained infection on Phaseolus lunatus, P. coccineus, P. angularis, Vigna sinensis, V. sesquipedalis, Soja max, and Dolichos lablab, but the organism failed to produce any symptoms on Lupinus polyphyllus, Pueraria hirsuta, Vicia faba, Pisum sativum var. arvense, and Melilotus alba.

Corynebacterium michiganensis. This pathogen has received considerable attention in recent years. Smith (1911) who first

described it on tomato believed it also occurred on the potato but he had no conclusive evidence to prove it. Stapp (1930) reported artificial infection on Pisum sativum and Phaseolus vulgaris but not on Vicia faba, Soja hispida and Pelargonium zonale. He could not obtain any infection on potato. Orth (1937) found Solanum humboldtii and S. pruniforme to be susceptible to C. michiganensis but Solanum racemigerum and S. racemiflorum were fairly resistant. McNew (1941) reported Hyoscyamus niger as a natural host of this organism. In his inoculation experiments, Lycopersicum chilensis, Nicotiana glutinosa and N. paniculata were very susceptible to this pathogen and Lycopersicum peruvianum var. tomatillo, Nicotiana sylvestris, N. rusbyi, and N. langsdorfii were moderately susceptible. On the other hand, Lycopersicum peruvianum, Phaseolus vulgaris, Nicotiana rustica, N. quadrivalvis, and N. auriculata were resistant and showed restricted infection. Datura stramonium, Pisum sativum, Solanum gilo, S. melongena, S. tuberosum, Phytolacca decandra, Nicotiana chinensis, N. tabacum (six varieties), N. alata, N. latissima, N. purpurea, N. colycotalis, and N. tomentosa failed to show any symptoms when inoculated with C. michiganensis. Ark (1944) reported Cyphomandra betacea, Solanum nigrum var. guineense, and Nicotiana glutinosa to be susceptible to this pathogen but not Nicotiana tabacum. Lycopersicum pimpinellifolium was slightly susceptible and inoculated plants were never killed.

Corynebacterium sepedonicum. Till 1913, this organism was reported on potato only. Spieckermann and Kotthoff (1914) reported tomato (Lycopersicum isculentum), and L. racimegerum to be susceptible to this pathogen but no mention was made of the symptoms or of reisolation. Solanum commersonii and S. cittrulifolium were also reported as susceptible hosts. Stapp (1930) observed local withering of leaves of young tomato transplants above and below points of inoculation. The organism was reported as mildly pathogenic to tomato. Pisum arvense, and Phaseolus vulgaris were also reported as slightly susceptible and the organism could be isolated from inoculated plants. Larson (1944) carried out extensive experiments on the inoculation of tomato and other members of the Solanaceae with C. sepedonicum and found that all commercial varieties of tomato were equally susceptible. Severe wilting and vascular necrosis was also produced on all the common eggplant (Solanum melongena) varieties and also in the wild, spiny, scarlet eggplant (S. integrifolium). In tests with following members of the Solanaceae, no symptoms developed and the organism was not recovered from inoculated plants: Atropa belladona, Browalia americana, Capsicum annum, Datura metel, D. meteloides, D. stramonium, Hyoscyamus niger, Lysium halimifolium, Nicandra physaloides, 15 species and varieties of Nicotiana, Nierembergia hippomanica, Petunia violacea, six species of Physalis, Salpiglossis sinuata, Schizanthus

wisetonensis and six species of Solanum.

Corynebacterium insidiosum. Jones and McCulloch (1926) who described this organism on alfalfa (Medicago sativa) reported that no symptoms developed on inoculated plants of red clover and several annual legumes (species not mentioned). Elliott (1930) reports alfalfa and white clover (Melilotus alba) as the only hosts of this pathogen. No other mention of the host range of this organism is available in literature and further work seems necessary.

Bacterium stewartii. Elliott (ibid.) reported Zea mays as the single host of this organism. Elliott (1935) reported Euchlaena mexicana, the annual species, to be susceptible to natural infection. Coix lachryma-jobi was reported a host of Stewart's wilt in greenhouse inoculation experiments (Poos and Elliott, 1936) and Ivanoff (1935) proved the susceptibility of Sorghum vulgare to the organism. Wellhausen (1938) reported Bacterium stewartii to be slightly infectious to beans and oats but tomatoes were not affected in greenhouse inoculations. Elliott and Poos (1940) carried out an extensive investigation on the host range of Bacterium stewartii and tested a number of plants of the tribe Tripsacaceae for their susceptibility to this organism. Besides Zea mays, Coix lachryma-jobi, Euchlaena mexicana, and E. perennis were the only plants that developed typical wilt symptoms on inoculation with Bacterium stewartii. On the other hand, Tripsacum dactyloides, T. lanceolatum, T. pilosum, T. latifolium, Sorghum

halepense, S. halepense sudanense, Saccharum officinarum, and Menisuris cylindrica were immune. Sorghum vulgare, Sclerachne punctata, Polytoca barbata, and Setaria glauca developed leaf symptoms in young seedlings only.

Pseudomonas solanacearum. Of all the wilt-bacteria, this species alone has the widest host range extending over several families of flowering plants. Amongst the more important hosts are potato, tomato, eggplant, tobacco, banana, and peanut. T. E. Smith (1939) carried out detailed field and greenhouse experiments on the host range of this pathogen and classified the plants tested into three classes, viz., species susceptible to natural and artificial infection (29 species), species susceptible to artificial infection but immune to natural infection (5 species), and species immune to both natural and artificial infection (56) species. He also suggested that sweet potato, cotton, watermelon, fireweed, Crotolaria striata, velvet bean, lima bean, soybean and cowpea be removed from the list of host plants and Xanthium pennsylvanicum, X. chinense, Physalis pruinosa, Aster pilosus, and Ambrosia trifida be added to it.

Erwinia tracheiphila. Elliott (1930) in her manual lists Cucumis melo, C. sativus, Cucurbita maxima, C. moschata and C. pepo as the natural hosts of this organism and Benincasa cerifera, Citrullus vulgaris, Cucumis anguria, C. odoratissima, Cucurbita californica, C. foetidissima, Echinocystis

lobata, and Sicyos angulatus were successfully inoculated in the greenhouse. Waldee (1945) found that this organism was pathogenic to cucumbers but not to plants of garden peas, potato, tobacco, and corn, and to green pear fruits, apple twigs, carrot slices, and potato tubers.

Experimental results.

In these experiments, the organisms studied were Xanthomonas campestris, X. lespedezae, Corynebacterium flaccumfaciens, C. michiganensis, and C. insidiosum. The plants on which inoculations were made were those that had not been previously tested for their susceptibility to one or more of the above organisms or whose reported susceptibility to one or more of the organisms was doubtful. All plants were grown in four inch pots, each pot holding four or five plants. Inoculations were made when the plants were about ten days old. The method of inoculation consisted of introducing a water suspension of the bacteria in the stem of each plant by means of a hypodermic syringe. Checks were inoculated with sterile water. Inoculated plants were incubated in a moist chamber for 48 hours before and after inoculation. Observations were made at three day intervals and the plants were discarded when a month old. The inoculations were repeated in every case.

Xanthomonas campestris. The winter stocks (Matthiola incana) has been reported as a host of this organism but the evidence on this point is conflicting. In the present experiments, seedlings of this plant, two weeks old, failed to show any symptoms of wilt in one month when inoculated with X. campestris. Cabbage seedlings (variety Early Jersey Wakefield) inoculated at the same time developed typical leaf symptoms in one week and four of the five inoculated plants had wilted in a month. These results would indicate that Matthiola incana is not a host of this organism.

Xanthomonas lespedezae. This organism failed to produce any symptoms of wilt on young plants of Phaseolus lunatus macrocarpus, Phaseolus aurens, Phaseolus acutifolius, and Stizolobium deeringianum in one month. Seedlings of Korean lespedeza, inoculated at the same time, showed typical symptoms in ten days.

Corynebacterium flaccumfaciens. No symptoms were produced on seedlings of Phaseolus lunatus macrocarpus, Phaseolus aurens, Phaseolus acutifolius, and Stizolobium deeringianum, whereas plants of golden cluster beans inoculated at the same time developed typical leaf symptoms in ten days.

Corynebacterium michiganensis. The ability of this organism to infect potato or otherwise has not been definitely proved. Young potato plants, a week old, were inoculated with

a virulent culture of the organism. One set of plants was inoculated by the hypodermic needle method and in another set the tops of plants were cut off with a flamed scalpel and an agar culture of the organism was applied to the cut ends. No symptoms of the disease appeared in a month's time whereas tomato seedlings (variety Bonny Best) inoculated at the same time developed typical cankers within 20 days.

No infection was produced on young seedlings of Solanum melongena, Capsicum annum, and Physalis pruinosa.

Corynebacterium insidiosum. Young seedlings of Phaseolus lunatus macrocarpus, P. acutifolius, Stizolobium deeringianum and White, White Dutch, Alsike, Black Medic, Crimson, Red, and Yellow Blossom clovers failed to show any symptoms of wilt in one month when inoculated with a virulent culture of the organism except in the case of white clover (Melilotus alba). Seedlings of alfalfa inoculated at the same time showed symptoms of wilt in three weeks.

Cross Inoculation Trials

The purpose of these trials was to determine whether any of the pathogens under study were able to invade the common hosts of the rest of the species of wilt-bacteria. Cross inoculations were made, therefore, with all the species, except Pseudomonas solanacearum ; P. solanacearum was not included

in the experiment as a highly virulent culture of the organism was not at hand.

The plants inoculated were cabbage (Early Jersey Wakefield), Korean lespedeza, beans (Golden Cluster), tomatoes (Bonny Best), potato, alfalfa, sweet corn (Golden Bantam), and cucumbers. About two weeks old plants were used for the inoculations. A water suspension of bacteria was used in every case and inoculations were made in the stems of the plants with a hypodermic syringe. All the plants were incubated in a moist chamber for 48 hours before and after inoculation and subsequently transferred to the greenhouse bench.

The cultures of the various species of wilt pathogens used in this experiment were of proved pathogenicity and had been recently re-isolated from diseased plants by the author. Table 13 shows the results.

The test was repeated twice with the same results. The results show that the species of wilt-bacteria tested seem to be unable to infect any of the plants tested except their own natural hosts by the method used. The only exception is Corynebacterium sepedonicum, which is able to infect tomatoes. These results are to be expected since the host range of the species, except Pseudomonas solanacearum, is not very wide.

Wellhausen (1938) reported a certain degree of infection of sweet corn by Xanthomonas campestris, Corynebacterium flaccumfaciens, C. michiganensis and C. insidiosum. Infection

Table 13. Results of cross inoculation trials.

Organism	Pathogenicity on							
	Cabbage	Lespedezae	Beans	Tomatoes	Sweet corn	Cucumber	Alfalfa	Potato
Xanthomonas campestris	+	-	-	-	-	-	-	-
Xanthomonas lespedezae	-	+	-	-	-	-	-	-
Corynebacterium flaccumfaciens	-	-	+	-	-	-	-	-
Corynebacterium michiganensis	-	-	-	+	-	-	-	-
Corynebacterium sepedonicum	-	-	-	+	-	-	-	+
Corynebacterium insidiosum	-	-	-	-	-	-	+	-
Bacterium stewartii	-	-	-	-	+	-	-	-
Erwinia tracheiphila	-	-	-	-	-	+	-	-

with these organisms was obtained only in very young seedlings of sweet corn. Infection of sweet corn, characteristic of Bacterium stewartii was never produced but only leaf symptoms and a little stunting was visible. He also reported Bacterium stewartii to be slightly infectious to beans but not to tomatoes. In the present experiments, B. stewartii failed to infect any other plant except sweet corn. Further, none of the other species produced any infection of sweet corn. Seedlings of all ages, from five days to three weeks old were inoculated, but in no case was infection visible. These conflicting results may be due to the differences in the virulence of cultures of B. stewartii, or the technique used.

The ability of C. sepedonicum to parasitize tomatoes was confirmed in these experiments.

An attempt is made in table 14 to summarize the extent of the host range of the wilt-bacteria. An examination of the table shows that none of the organisms are restricted to one host. The least number of hosts is two in the case of Corynebacterium insidiosum; this organism has not received much attention in recent years and it is safe to assume that more hosts for this organism will be found when further work is done. Most of the work on the host range of the wilt-bacteria has been in the last ten years or so and before that these organisms along with other phytopathogenic bacteria were supposed to be very restricted in their host range. Studies

Table 14. Extent of host range of the bacterial wilt pathogens.

Organism	Total no. susceptible hosts	Distribution of susceptible hosts	
		families	genera
Xanthomonas campestris	12	1	2
Xanthomonas lespedezae	10	1	1
Corynebacterium flaccumfaciens	0	1	4
Corynebacterium michiganensis	16	2	7
Corynebacterium sepedonicum	9	2	4
Corynebacterium insidiosum	2	1	2
Bacterium stewartii	4	1	3
Pseudomonas solanacearum	109 (Elliott, 1930)	27	70
Erwinia tracheiphila	13	1	7

on the host range of any phytopathogenic organism can never be too extensive or "complete", since all that is attempted by any worker is testing of available species, particularly those belonging to the same family as the common host of the pathogen. Further work may find other new unknown hosts.

DISCUSSION

An attempt is made in table 15 to record pertinent information on the growth reaction and cultural response of the species of wilt bacteria studied. It will be seen from table 15 that the pathogens fall into two distinct groups. Group 1, composed of Xanthomonas campestris, X. lespedezae, Bacterium stewartii, and Pseudomonas solanacearum, is characterized by the biochemical activity of its members; the cabbage and Lespedeza pathogens especially are very active. They hydrolyze starch, liquefy gelatin, proteolyze milk and produce indole. Bacterium stewartii and Pseudomonas solanacearum do not possess these characteristics; P. solanacearum differs from the rest in its ability to reduce nitrates and inability to produce hydrogen sulphide. In fact it is the only species of all the wilt bacteria that has these characters.

All the four pathogens in this group multiply rapidly and have a wide growth temperature range. The pathogens in group 2, on the other hand, grow much more slowly. Inorganic nitrogen is utilized by all members of group 1 and they can also utilize organic nitrogen in the form of amino acids, although the different species in this group show some variation in this respect. This may have some bearing on their host relations. For example, Pseudomonas solanacearum can utilize β- alanine, tyrosine, asparagin, and glutamic

Table 15. Comparisons between the growth reaction and cultural response of some wilt producing bacteria.

Bacterial pathogens	Gram stain	Motility	Gelatinlique.	Litmus milk	Indole	Starch hydro.	H_2S	Nitrate reduct.
Group 1								
Xanthomonas campestris	-	+	+	proteo.	+	+	+	-
Xanthomonas lespedezae	-	+	+	proteo.	+	+	+	-
Bacterium stewartii	-	-	-	no action	-	-	+ weak	-
Pseudomonas solanacearum	-	-	-	alkali	-	-	-	+
Group 2								
Corynebacterium flaccumfaciens	+	+	-	proteo.	-	-	+	-
Corynebacterium michiganensis	+	-	+	curd	-	-	+	-
Corynebacterium senedonicum	+	-	-	reduct.	-	+ weak	+	-
Corynebacterium insidiosum	+	-	-	reduct.	-	-	+	-

Nitrate reduct.	Growth-temp. range °C.	Reaction from carbon sources in peptone basal medium				Utilization of citrate	Utilization of inorganic nitrogen	β-d wit dex
		Dextrose	Sucrose	Lactose	Glycerol			
-	15-40	alkali	alkali	alkali	alkali	+	+	-
-	15-40	alkali	alkali	alkali	alkali	+	+	-
-	15-40	acid	acid	acid	acid	-	+	+
+	20-40	alkali	alkali	alkali	alkali	+	+	+
-	15-40	acid	acid	acid	acid	-	-	
-	20-30	acid	acid	acid	acid	-	-	
-	15-25	acid	acid	acid	alkali	-	-	
-	15-25	acid	acid	acid	acid	-	-	

Utilization of organic nitrogen with and without dextrose as source of carbon

β-alanine		Tyrosine		Cystine		Lysine		Aspartic acid		Asparagin		Glutamic acid	
with dex.	without dex.	with dex.	without dex.	with dex.	without dex.	with dex.	without dex.	with dex.	without dex.	with dex.	without dex.	with dex.	without dex.
−	−	+	−	+	+	+	+	+	+	+	−	+	+
−	−	+	−	+	−	+	−	+	−	+	−	+	+
+	−	+	−	+	−	+	−	+	−	+	−	+	+
+	+	+	+	+	−	+	−	+	−	+	+	+	+
	−		−		−		−		−		−		−
	−		−		−		−		−		−		−
	−		−		−		−		−		−		−
	−		−		−		−		−		−		−

acid as sole sources of carbon and nitrogen, a characteristic that might be correlated with the wide host range of this pathogen. Further, this species differs from the rest in that it is not strictly limited to the vascular system of its host, but is able to destroy the parenchyma. It is felt that the ability of this pathogen to produce nitrites from nitrates may have bearing on this phenomenon since nitrites are very toxic to plant cells and their production may enable the parasite to break out into the parenchyma. Certainly, nitrites may well be one of the causes of the wilting of the host.

Bacterium stewartii is not very active in its enzymatic behaviour but can utilize a large number of organic nitrogen compounds if sugar is present; the role of sugar in the metabolism of this pathogen is very striking. In its absence, only glutamic acid is utilized but most of the amino acids serve as sources of nitrogen if carbon is supplied in the form of a sugar.

The members of Group 1 seem to be rather primitive organisms when compared with those in Group 2, which is composed of the Corynebacteria. These pathogens are slow growers, have narrow limits of growth temperature, and a low biochemical activity; gelatin liquefaction is not very strong, starch is not usually hydrolyzed, indole is not produced and proteolysis in milk does not occur. Corynebacterium flaccumfaciens is more active biochemically than the rest as shown by

its ability to proteolyze milk and liquefy gelatin. None of the four Corynebacteria can utilize inorganic nitrogen and only a very small number of amino acids are utilized if a sugar is available as carbon source. On the other hand, peptone serves as a source of carbon and nitrogen for all the species in this group. It seems, therefore, that the nutritive requirements of this group are very complex and growth accessory factors--"nutrilites" and vitamins--may be necessary for their development. This characteristic, coupled with their lack of enzymatic activity, might explain why these organisms are vascular rather than parenchymatous. The biochemical inactivity of these organisms can further be correlated with their pathogenesis; none of the wilts caused by the Corynebacteria are as intense in their manifestations as the ones where the causal agents belong to Group 1, the active parasites. For example, bean wilt caused by Corynebacterium flaccumfaciens and tomato wilt caused by C. michiganensis are never as destructive as the "black rot", Stewart's wilt, and wilt of potatoes and tomatoes. It is well known that many plants may wholly or partially recover.

Still further, C. sepedonicum develops slowly in its host, so much so that the presence of the pathogen in diseased tissues is difficult often to demonstrate.

It is surprising why members of Group 1, very active biochemically, are vascular parasites. The Xanthomonas

species are particularly noteworthy in this respect. The genus Xanthomonas is made up of species which have the same biochemical activity as the two wilt producers in that genus, but the symptoms produced by members of this genus are "leaf-spots" and "blights". The cabbage and lespedeza pathogens, on the other hand, produce vascular necrosis. The nutritional requirements of this group are also simple and special growth accessory factors are not necessary as in the members of Group 2. Starr (1946) studied the "minimal nutritive requirements" of 30 species of Xanthomonas (including X. campestris and X. lespedezae), and found that all the species could grow on a simple basal medium containing ammonium chloride, glucose, and salts and growth accessory factors such as methionine, glutamic acid and nicotinic acid were not necessary. The only exceptions were X. pruni which required nicotinic acid to support its growth, and X. hederae and X. transluscens, which required methionine as a growth accessory factor. The results of the present study confirm these observations with respect to X. campestris and X. lespedezae in so far as these two species can grow in an inorganic nitrogen medium. Why is it then that in such a homogeneous group of organisms like species of Xanthomonas, only a few are vascular parasites? The answer clearly does not lie in the "minimal nutritive requirements" of these organisms or their enzymatic activity. It is possible that the living cell in leaves of the host

plant elaborate one or more chemicals that either kill the invading bacteria or inhibit their growth with the result that only small necrotic lesions result when invasion occurs through the stomata, while this does not happen in the vascular system which is composed of non-living elements. The occurrence of small, localized lesions on cabbage as a result of stomatal invasion by Xanthomonas campestris supports this hypothesis. A somewhat similar phenomenon is found in black-stem rust caused by Puccinia graminis tritici, where in moderately resistant varieties the killing of host cells adjacent to the substomatal vesicle releases substances which kill the invading fungus hyphae (Allen, 1923). Recently, Kohman (1947) has shown that a distillate of onions, removed from contact with enzymes, undergoes a change and yields dinitro-phenyl hydrazone, which has distinct bactericidal properties. Pederson and Fisher (1944) have shown the bactericidal properties of cabbage juice.

It is interesting to speculate on the existence of antibiotic phenomena in the host relations of the vascular pathogens. In the case of the "leaf-spot" and "blight" organisms, it is possible that the bacteria themselves elaborate some chemicals, not necessarily enzymes, which enable them to kill the living cells in the leaf, a property that might be absent in the vascular pathogens. The existence of such a phenomenon cannot be demonstrated unless specific

microchemical tests are developed and utilized. One of the animal pathogens, such as the tuberculosis organism elaborates some 300 different chemical compounds (in Kohman, 1947); it is possible that the plant pathogens, too, behave similarly.

Some light may be shed on the host relations of the wilt bacteria by recalling host relations of the Fusarium wilts. Fusarium conglutinans Wr. parasitizes cabbage and other crucifers but no other hosts in related families. Xanthomonas campestris is very much similar in its host range. On the other hand, Fusarium vasinfectum Atk. and F. lini Bolley are very restricted. These Fusaria have simple nutritional requirements and can utilize inorganic nitrogen; they also produce most of the common enzymes and F. vasinfectum even produces cellulase. These parasites, although they have a less specific nutritional requirement than any of the wilt bacteria, are highly specific. Parasitism is a phenomenon of nutrition of the pathogen, after all, and it would seem therefore that the answer to vascular parasitism of the wilt bacteria lies in their specific nutritional requirements. In the last analysis one is forced to conclude that parasitism in the wilt bacteria is dependent on nutrilites supplied by the host or pathogen.

Before further work is done in this problem, it would be worthwhile to find out whether the species of wilt bacteria are really unit or group species. The results on the amino acid

utilization by strains of Corynebacterium michiganensis indicates that this species is made up of strains. Similar variations exist in Bacterium stewartii. It is felt that with the development of an appropriate technique for measuring the virulence of these bacteria and by the use of differential hosts, as is done in the rusts and powdery mildews, most of the bacterial plant pathogens could be shown to be very variable and composed of strains. The existence of such a situation in Xanthomonas translucens and the root-nodule organism, Rhizobium leguminosarum is well known. It should prove illuminating to study the growth response of these strains comparatively on the organic nitrogen compounds. In other words, further progress on this problem must combine strain isolation in terms of host reaction and the growth response of the different variants in each species of the wilt bacteria.

SUMMARY

Thirty-two isolates of nine species of wilt-producing plant pathogenic bacteria belonging in the genera Xanthomonas, Pseudomonas, Corynebacterium, Erwinia and Bacterium were studied comparatively for their cultural behaviour and host relations.

All the species were rod shaped, non-sporulating, non-acid fast organisms. The Corynebacteria were Gram positive while the rest were Gram negative; the Corynebacteria (with the exception of C. flaccumfaciens) and Bacterium stewartii were non-motile but the rest were motile.

All the isolates produced a yellowish, slimy growth on the media used; exceptions were Pseudomonas solanacearum, was dirty-white turning brown, Corynebacterium sepedonicum, white and C. insidiosum, bluish-black.

Xanthomonas campestris, X. lespedezae, Pseudomonas solanacearum, Bacterium stewartii, and Corynebacterium flaccumfaciens had a very wide optimum growth-temperature range of 15° - 40° C.; the rest of the species had narrower limits of temperature, 20° - 30° C., for optimum growth.

Xanthomonas campestris and X. lespedezae were active liquefiers of gelatin and also hydrolyzed starch. Corynebacterium hydrolyzed starch slightly but did not liquefy gelatin. Some isolates of C. michiganensis and C. flaccum-

faciens liquefied gelatin moderately but none hydrolyzed starch. All the rest of the species neither attacked gelatin nor starch.

Hydrogen sulphide was produced by all the species except Pseudomonas solanacearum, when tested by the lead-acetate strip method. The production of hydrogen sulphide by Corynebacterium flaccumfaciens, C. michiganensis, C. insidiosum and Bacterium stewartii was demonstrated for the first time.

Only some isolates of Xanthomonas campestris and all of X. lespedezae produced indole weakly.

The Xanthomonas species and Corynebacterium flaccumfaciens proteolyzed milk. Corynebacterium insidiosum and Bacterium stewartii produced slight acidity while Erwinia tracheiphila produced no change in litmus milk; Corynebacterium michiganensis curdled it and Pseudomonas solanacearum produced a distinct alkaline reaction.

Pseudomonas solanacearum alone reduced nitrate to nitrite and further utilized it; the rest of the species did not reduce nitrate.

All the species were Voges-Proskauer and Methyl Red negative. The two Xanthomonas species and Pseudomonas solanacearum utilized citrate.

Only Bacterium stewartii and Pseudomonas solanacearum could utilize asparagin as sole source of carbon and nitrogen.

The Corynebacteria produced an acid reaction in a peptone

basal medium with all the carbon compounds tested. They failed to grow in a synthetic nitrogen media with carbon compounds as the source of energy. The Xanthomonas species and Pseudomonas solanacearum produced an alkaline reaction with all the carbon compounds in a peptone basal medium; Bacterium stewartii produced an acid reaction which reverted to neutrality with age.

In a synthetic basal medium with ammonium phosphate as the source of nitrogen, Xanthomonas campestris and X. lespedezae utilized a large number of carbon compounds including acetic and citric acids. Pseudomonas solanacearum utilized only a few of these compounds while Bacterium stewartii was intermediate between Pseudomonas solanacearum and the Xanthomonas species.

Very striking differences were exhibited by the nine species of wilt-bacteria in their utilization of amino acids as a source of nitrogen and/or carbon in a synthetic mineral basal medium. The Corynebacteria were very inert in this respect; glutamic acid was utilized as a source of nitrogen by all species in the presence of dextrose as a source of carbon. There were differences between highly virulent and slightly virulent strains of Corynebacterium michiganensis; the highly virulent ones utilized aspartic and glutamic acids as sole source of carbon and nitrogen, while the slightly virulent ones did not. The virulent ones utilized a larger number of amino acids in the presence of dextrose than the slightly virulent ones.

Wounds are necessary for host invasion by the wilt bacteria in most cases, though some of them like Xanthomonas campestris and Corynebacterium michiganensis can penetrate the host through natural openings like hydathodes and stomata.

Majority of the parasites invade the xylem principally; exception is Corynebacterium michiganensis which parasitizes the phloem and causes cankers on the stems.

It was shown that Xanthomonas campestris and X. lespedezae can invade their hosts through stomata on the leaf, but the resulting lesions remain small and the organisms do not progress further.

The physiological characters of Corynebacterium flaccumfaciens justify its inclusion in that genus. It is felt that the Stewart's wilt pathogen deserves to be placed in a new genus in the Pseudomonadeceae, but no recommendations are made since it seems that more isolates of this organism should be examined.

The winter stock (Matthiola incana) was not susceptible to the strains of Xanthomonas campestris used in this study. Corynebacterium michiganensis failed to cause any symptoms in potatoes.

In cross-inoculation trials where all the common hosts of the wilt-bacteria (except Pseudomonas solanacearum) were used, the pathogens did not produce symptoms in any plants, but their common hosts; the only exception was Corynebacterium sepedonicum which could invade the tomato and the potato.

The present study indicates that the wilt causing bacteria can be divided into two groups based on their differential growth response, namely their enzymatic activity, nitrogen utilization and growth temperature range. Definitely these eight bacterial plant pathogens are not as heterogeneous in growth reaction as their systematic grouping suggests. Group 1 comprises of four species as follows: Xanthomonas campestris, X. lespedezae, Pseudomonas solanacearum, and Bacterium stewartii, and Group 2 four also, namely, Corynebacterium flaccumfaciens, C. michiganensis, C. sepedonicum, and C. insidiosum. The growth reaction of Group 2 shows considerable specificity on the nitrogen containing amino acids which suggests that the growth of these bacteria in the host is dependent on specific nutrilites elaborated by the interaction of the host and parasite. What these may be can only be determined by strain isolation based on differential host reaction and an intensive study of their growth response.

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